SEABIRD DIGESTIVE PHYSIOLOGY IN RELATION TO FORAGING ECOLOGY

by

Susan Jackson

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Department of Zoology

Faculty of Science

University of Cape Town

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For Phil

And for the Jacksons: Jill, Jane, Paul and Jay

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ABSTRACT

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This study addresses the question: are seabirds digestive opportunists or specialists? The extent of specialization in seabird digestive processes to different diets and foraging methods, is investigated experimentally. Gut passage rates of different food types of tested *in vitro* digestibility may reflect dietary specialization, with favoured prey types excreted more rapidly than less frequently encountered prey. Mean retention times of solid digesta are significantly correlated with foraging trip duration, and with gut length. Gut length and volume in turn scale with body mass. Assimilation efficiencies of various dietary components are not predictable purely on the basis of food composition, and show a high degree of inter- and intraspecific variability. Energy assimilation efficiency does not reflect dietary specialization, and may be maintained at approximately 75% regardless of diet. Assimilation efficiency is, however, temporarily elevated in energetically-stressed birds, such as penguins that have recently completed moult.

Digestive specializations are reflected in seabirds' abilities to assimilate substances specific to certain prey organisms. Unlike most terrestrial vertebrates, seabirds are able to digest wax esters, compounds important in marine food webs. Procellariiforms exhibit unique gastric adaptations facilitating extended foraging trips and efficient transport of food to their young, both important advantages for predators exploiting patchy and unpredictable food resources. Seabirds which naturally feed on crustaceans secrete the specific enzyme chitinase from their gastric mucosae, permitting digestion of the chitinous exoskeleton of the prey. The ability to secrete this enzyme is probably a retained ancestral trait rather than a newly evolved one, and may have been been lost by seabirds that do not prey on crustaceans.

Differences between penguins and procellarids reflect unique adaptations to the

different foraging techniques employed by these two families. The synthesis of the thesis explores the adaptive significance of gut passage rate and allometry of the gut in relation to the two predominant foraging techniques employed by seabirds: long-distance aerial soaring and subsurface swimming. Scaling of seabird gut size may play an important role in the interplay between metabolic rate, the energy demands of foraging, and digestive physiology. The allometric approach taken here is potentially useful for studies of seabird digestion, and has application in studies linking the evolution of avian body size, and foraging ecology.

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GENERAL INTRODUCTION

For a seabird to survive and reproduce in the marine environment, it must locate prey that is both spatially and temporally unpredictable. Breeding birds must then transport food over sometimes long distances to feed their chicks, at the same time as satisfying their own energy needs. These dual constraints may result in energy limitation during breeding, as is suggested by the life-history attributes of many pelagic seabirds (Lack, 1968; Ricklefs, 1983). The survival of adult seabirds during the non-breeding season is less likely to be threatened by lack of food (Ricklefs, 1983). Seabirds display a wide array of morphological adaptations for efficient prey capture (Ashmole, 1971; Brown *et al.*, 1978), many of which are related to body size (Ashmole, 1968; Pennycuick *et al.*, 1984). The allocation of energy once assimilated has been well studied (see Whittow and Rahn, 1984 for a review). Digestion forms a crucial link between the ingestion of energy and its consumption, and comparative studies of the digestive physiology of species with different trophic niches should complement studies of seabird feeding ecology. There is considerable evidence for dietary segregation amongst breeding (Ashmole and Ashmole, 1967; Croxall and Prince, 1980; Adams and Brown, 1989) and non-breeding seabirds (Ainley and Sanger, 1979; Sanger, 1986), implying a degree of feeding specialization.

Digestive specializations have been documented in seabirds that breed at high latitudes: several species of procellariiforms are efficient at digesting and assimilating wax esters, complex lipids important as energy reserves in marine food webs (Obst, 1986; Place and Roby, 1986; Roby and Place, 1986; Place *et al.*, 1989). With the premise that the acquisition of energy is probably the single most important selective pressure operating on adult seabirds, this thesis asks the question: if food resources are unpredictable, are the digestive abilities of seabirds conservative, reflecting dietary opportunism, or are there interspecific digestive specializations which reflect interspecific dietary specializations?

The thesis also aims to increase our understanding of interactions between feeding ecology and physiology by exploring digestive adaptations in pelagic

seabirds that are operating under the constraints of two different foraging methods: long-distance flight and subsurface swimming. Gliding flight is highly developed in the Procellariiformes (petrels and albatrosses), and is an energy-economical way of searching for prey over large areas of the ocean surface. One might expect gut capacity in flying seabirds to be constrained by the necessity of minimizing weight carried (Sibly, 1981). The relationship between body mass and the lifting power of avian muscles probably sets an upper limit to the size which an albatross can attain, and still lift a food load large enough to meet its own energy requirements and those of its chick (Ricklefs, 1983; Pennycuick, 1975, 1984). In contrast, penguins are not constrained by the need to minimize their total body weight because they employ subsurface swimming, a foraging method less economical than gliding flight but more efficient than surface swimming (Baudinette and Gill, 1985). The advantages of this method are twofold: penguins are able to forage in three dimensions, increasing their probability of encountering prey, and they can attain larger body sizes than can flying seabirds, thereby decreasing mass-specific heat loss and improving their chances of survival in winter at extreme latitudes.

The ecological significance of stomach oil formation in the procellariiforms has been well reviewed (Warham *et al.*, 1976; Place *et al.*, 1989), and this digestive adaptation is in part a mechanism for reducing the weight carried by foraging birds without sacrificing energy returns from digestion (Ricklefs, 1983). By comparing gut passage rates of solid and aqueous digesta in procellariiforms and penguins, I investigate the influence of flight-related constraints on digestive tract structure and function.

Experimental approach and structure of the thesis

Chapter 1 supplies data on the intrinsic digestibilities (in a standard pepsin solution) of the three prey categories used in later feeding experiments: fish, squid and crustaceans. These data provide the basis for comparison with *in vivo* digestibility and gastro-intestinal passage rates of the same foods. The three food types were chosen to embrace the most important prey categories eaten by seabirds

in the wild. Chapter 2 describes a novel technique for investigating gastric digestion rates in seabirds without resorting to lethal methods, or to relatively stressful stomach-pumping. The limitations of the technique are discussed, and gastric digestion rates in one species of procellariiform are described.

By means of feeding experiments with captive Cape Gannets, procellariiforms and penguins at their breeding sites, Chapter 3 investigates the two major issues addressed by the thesis (see above). The degree and extent of specialization of seabird digestive systems in relation to natural diet is investigated by comparison of gut passage rates of three prey categories between species with differing trophic niches. Mean gut retention times of the dry fraction of digesta, and gut dimensions, are compared between flying seabirds and penguins, to test the prediction that flying seabirds "process" food faster to reduce weight carried. Chapter 4 supplies data on the chemical composition and energy values of the food types used, and assesses whether or not assimilation efficiencies are predictable solely on the basis of food composition.

Chapters 5 and 6 investigate seabirds' abilities to digest and assimilate substances of potential importance in marine food webs. Chapter 5 deals with wax ester assimilation, and Chapter 6 with chitin digestion. By comparison of representative procellariiforms, penguins and a sulid, differences between major taxonomic groups are assessed. Chapter 7 describes the ecological implications of seabird body size, a parameter which has a profound influence on the structural and function of the digestive tract. This chapter draws on data from Chapters 3 and 4 and from the literature, and provides a conceptual framework for integrating the preceding chapters.

Each of the thesis chapters is written as a discrete paper, to facilitate communication of ideas. The advantages of this structuring hopefully outweigh the disadvantages of occasional duplication in methodological descriptions and reference lists. Three of the thesis chapters are published or in press, and the coauthors of these chapters are fully acknowledged both on the title pages of the

chapters and in the acknowledgements section. Cross-references between chapters are in the form of references to chapters rather than to papers. Two previously published papers which are pertinent to the discussions of Chapters 2, 3, 4 and 7, but separate from the body of data presented in the thesis, are included as Appendices 1 and 2.

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CHAPTER 1

GASTRIC DIGESTION IN MARINE VERTEBRATE PREDATORS: IN VITRO STANDARDS

With D.C. Duffy and J.F.G. Jenkins, *Functional Ecology* 1:287-291 (1987)

SUMMARY

In vitro studies of digestion rates of various prey types provide standards of digestibility that are useful in studies investigating digestive adaptations in marine predators. Total digestion of crustacean muscle takes a mean of 17 hours, whereas breakdown of fish and squid muscle takes between 8 and 9.5 hours. Pepsin does not break down the exoskeletons of whole crustaceans such as prawns *Penaeus indicus* and Antarctic Krill *Euphausia superba*. The ranking of *in vitro* digestibility of various prey types is the same as that of published data from *in vivo* experiments on seabirds. Previously-frozen samples are digested more rapidly than fresh samples in pepsin. Agitation increases digestion rate.

INTRODUCTION

There are interspecific differences in the abilities of marine vertebrates to digest and assimilate various prey types (Bigg and Fawcett, 1985; Heath and Randall, 1985; Wilson *et al.*, 1985; Laugksch and Duffy, 1986; Jackson and Ryan, 1986). Determination of digestion rates of a range of prey under standardised, *in vitro* conditions can indicate how much of this variation is attributable to differences in prey tissue, and/or to differences in the digestive ability of predators. Here it is shown how this approach can be applied to an investigation of digestive adaptations in marine predators such as seabirds.

Although some seabirds can be stomach-pumped at increasing intervals after food ingestion, using a non-lethal sampling technique (cf Wilson, 1984; Wilson *et al.*, 1985), sampling of marine mammals may be destructive (eg: Bigg and Fawcett, 1985, Jackson and Ryan, 1986). This is undesirable, particularly when the animals studied are rare. This study aims, therefore, to establish *in vitro* standards for comparison with *in vivo* digestion experiments that will lessen the need for destructive sampling. The results may also indicate potential biases in diet studies on predators for which differential digestion rates of prey are not yet known.

MATERIALS AND METHODS

The experimental methods resemble those of Bigg and Fawcett (1985). Between seven and ten samples of prey tissue were lowered in plastic bags with 8 x 5 mm mesh, into 240 ml of a digestive solution. The solution comprised 0.5% HCl, 0.6% Na_2CO_3 and 1% pepsin (B.D.H. Chemicals Ltd, "Pepsin A" powder, activity 1 Anson unit per gram). The HCl concentration was adjusted to give the solutions an initial pH of 1.25 - 1.35, within the range of 0.9 - 2.9 described by van Dobben (1952) for the gastric pH of Great Cormorants *Phalacrocorax carbo* (Linnaeus). The beakers were maintained in water baths at 38 - 40°C, approximately the deep body temperature of the large marine birds and mammals (Calder and King, 1974).

At one-hour intervals, the samples were lifted out of the beakers, drained of all drops of digestive solution clinging to the mesh, and weighed with a Pesola spring balance accurate to the nearest 0.5 g. The pH of the solution in each beaker was measured with a Beckman expanded-scale pH meter before replacing the specimen: throughout all trials pH remained between 1.25 and 1.75. Weighings were repeated at hourly intervals until mean mass loss of all samples per interval was less than 5% of the original masses of the samples. Thereafter, measurements were made every two hours, or, in the case of intact crustaceans (see below), less frequently.

Initially, the effect of previous freezing on the digestion rates for four prey types was investigated. Ten samples each of intact Cape Anchovy *Engraulis capensis*, Rock Lobster *Jasus lalandii* tail muscle, and pieces of hake *Merluccius* sp. and squid *Loligo vulgaris reynaudii* muscle were frozen, then thawed, and placed in pepsin. Ten fresh controls were digested in the same way.

The effect of simulated movement or mechanical breakdown on rates of digestion of the four prey types used above, was then investigated. Ten beakers containing samples of each prey type were supported by a platform agitated 24 times per minute in one horizontal plane, with an 8-cm range of movement. Ten controls were digested in stationary beakers.

Wilcoxon's one-tailed pairwise U-test was used to test the prediction that freezing and/or agitation increase the rapidity of tissue digestion. Mean times at which samples had lost 25, 50, 75 and 100% of their original wet mass, were compared within each prey type between specimens subjected to the two treatments described above.

Finally, the digestibilities of intact Cape Anchovy and Antarctic Krill *Euphausia superba*, pieces of hake liver, hake muscle, squid mantle muscle, Rock Lobster tail muscle (without exoskeleton), and pieces of prawn *Penaeus indicus* tail (with exoskeleton), were compared. Anchovy, squid and krill represent the three major prey categories eaten by the seabirds used in the feeding experiments described in Chapters 2, 3 and 4. Hake is important in the diets of Cape Gannets and White-

chinned Petrels in the southern Benguela region (Cooper, 1984; Jackson, 1988). Rock Lobster muscle was used for comparison with the other two crustacean foods, which were enclosed by exoskeleton. Pieces of muscle and liver weighed 4.0 - 5.5 g, and were cut to the same linear dimensions and shapes to minimise variations in surface area to volume ratios. Limited availability of intact animals precluded selection of specimens of the same sizes, so weights ranged from 3.0 - 9.0 g. Seven replicates of prawn tail and Antarctic Krill were used, and all other prey types were represented by ten replicates. All specimens were agitated 24 times per minute during digestion. After 96 hours, prawn and krill exoskeletons showed no signs of disintegration, although all muscle tissue had been digested. What remained of the specimens was weighed, and dried at 40°C for three days before reweighing and determination of chitin content for comparison with values for undigested specimens. The methods used in the chitin analyses are described in Chapter 6.

Times to digestion of 25, 50, 75 and 100% of intact anchovy and krill, hake liver and muscle, squid and Rock Lobster muscle, and prawn tails, were compared using a non-parametric single-factor analysis of variance incorporating the Kruskal-Wallis test (Dunn, 1964). All specimens used in this comparison had been previously frozen, because food used in the experiments described in following chapters had been stored frozen. The specimens were agitated during digestion.

A separate analysis was used to facilitate direct comparison of *in vitro* digestion rates with *in vivo* gastrointestinal passage rates for seabirds (Chapter 3). Mean retention times of samples of each prey type were calculated using the formula:

$$t = \sum_{i=1}^{n} m_i t_i \Big/ \sum_{i=1}^{n} m_i$$

where t equals the mean retention time of a given unit (in this case, wet mass) of food, and m_i the mass of food digested at time interval t_i after commencement of digestion (Warner, 1981). Again to facilitate comparison with *in vivo* data, two-hourly time increments were used in the calculations. For simplicity, mean

retention times were only calculated for food types actually used in the *in vivo* feeding experiments (Chapter 3). A single value was calculated for each specimen of each prey type, with the maximum time interval taken as the time to complete digestion. In the case of prawn tails and krill, the maximum time interval was taken as the time to digestion of all tissue other than exoskeleton ie: the time of termination of the experiment. Results of these analyses were compared between foods using the non-parametric single factor analysis of variance mentioned above. All differences mentioned in the results section are significant.

RESULTS

Effects of freezing and agitation on digestion rates

Digestion of 25% of intact anchovy was faster for fresh than for frozen specimens (U-values and levels of significance are given in Fig. 1.1a). Fresh squid muscle was digested more rapidly than was frozen tissue at all but the final stage (Fig. 1.1b). Frozen hake (Fig. 1.1c) and Rock Lobster muscle (Fig. 1.1d) lost mass more rapidly than did fresh samples at all stages.

For all four food types, digestion rates for agitated samples were significantly faster than corresponding values for stationary samples (U-values and significance levels are given in Figs 1.2a, b, c, and d).

Digestion rates of different food types

Digestion rates of agitated samples of different food types varied greatly. Hake liver was digested fastest, followed by hake muscle, intact anchovy, squid muscle, and Rock Lobster muscle (Figs 1.3a and b, Table 1.1). Squid muscle initially gained mass, but subsequently was digested at a rate similar to that of fish. Mean time for total dissolution of all samples of squid and fish was the same (11 hours), whereas Rock Lobster muscle was completely digested only after a mean time of 16.4 hours. Although the initial digestion of krill was more rapid than that of squid (Table 1.1), the indigestibility of crustacean exoskeleton reversed this difference in the later

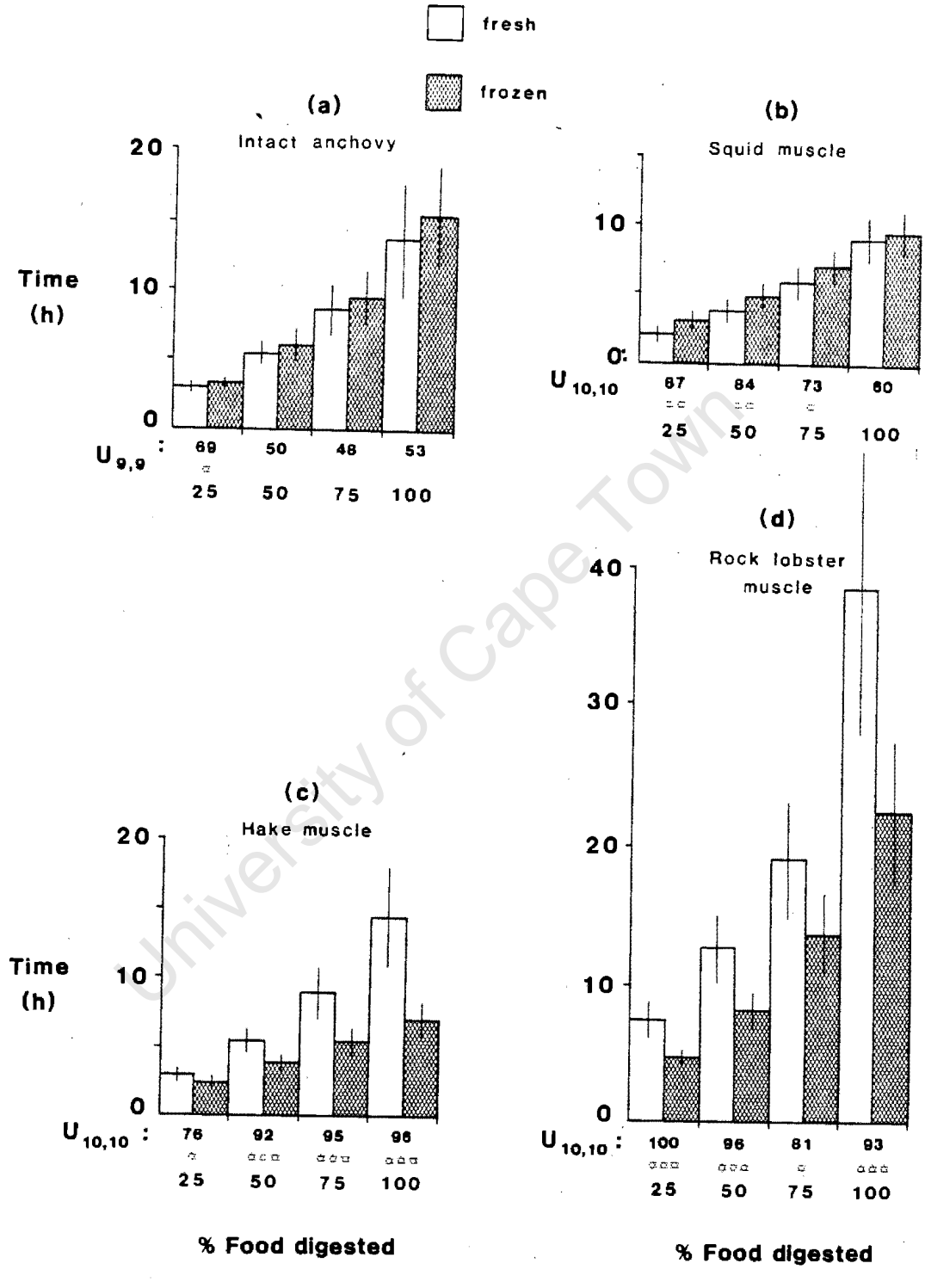

Figure 1.1. Time elapsed for digestion of 25, 50, 75 and 100% of fresh and previously frozen food samples. Vertical bars represent 1 S.D. Significant differences between fresh and frozen samples for each time interval, judged by Wilcoxon's one-tailed pairwise U-test, are indicated as follows; *: $P < 0.05$, **: $P < 0.005$, ***: $P < 0.001$.

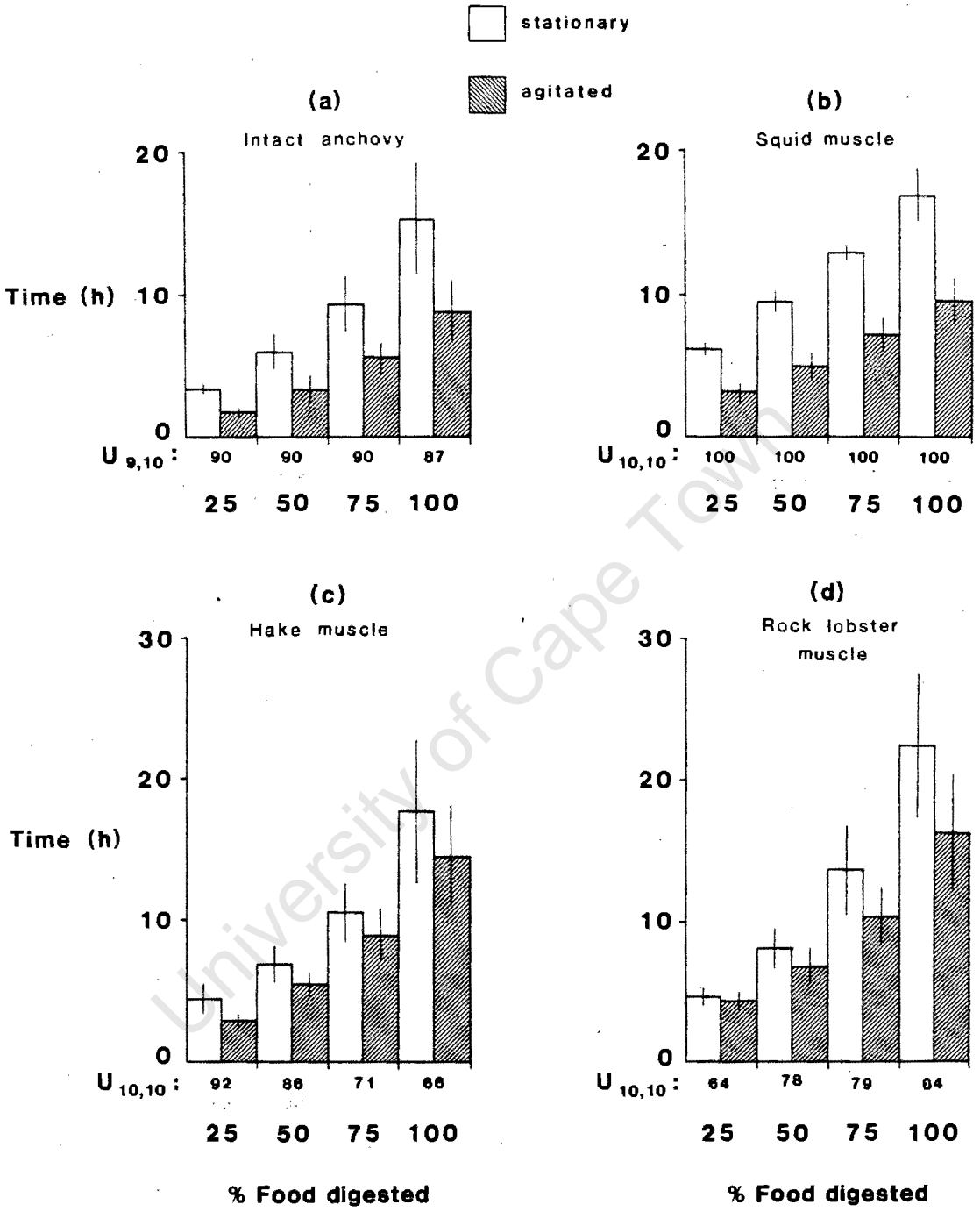

Figure 1.2. Time elapsed for digestion of stationary and agitated food samples. Notation as for Figure 1.1.

Table 1.1. Times to loss of 25, 50, 75 and 100% of original sample mass for different food types. Figures in parentheses = 1 S.D. "nd" = no data.

Mean % mass of samples digested	Mean time elapsed (h) for digestion of given proportions of different foods						
	Prey type						
	Hake liver	Intact anchovy	Hake muscle	Squid muscle	Rock Lobster muscle	Intact krill	Prawn tails
25	1.57 (0.33)	1.70 (0.24)	2.33 (0.48)	3.07 (0.69)	4.35 (0.69)	3.76 (2.45)	26.46 (11.23)
50	2.60 (0.60)	3.35 (0.96)	3.85 (0.65)	4.97 (0.91)	6.79 (1.25)	13.23 (5.54)	50.27 (16.50)
75	4.15 (1.72)	5.53 (1.13)	5.44 (1.08)	7.09 (1.23)	10.39 (2.13)	35.71 (12.38)	60.22 (14.75)
100	6.50 (3.06)	8.70 (2.21)	7.70 (1.42)	9.50 (1.65)	16.40 (4.09)	nd	nd

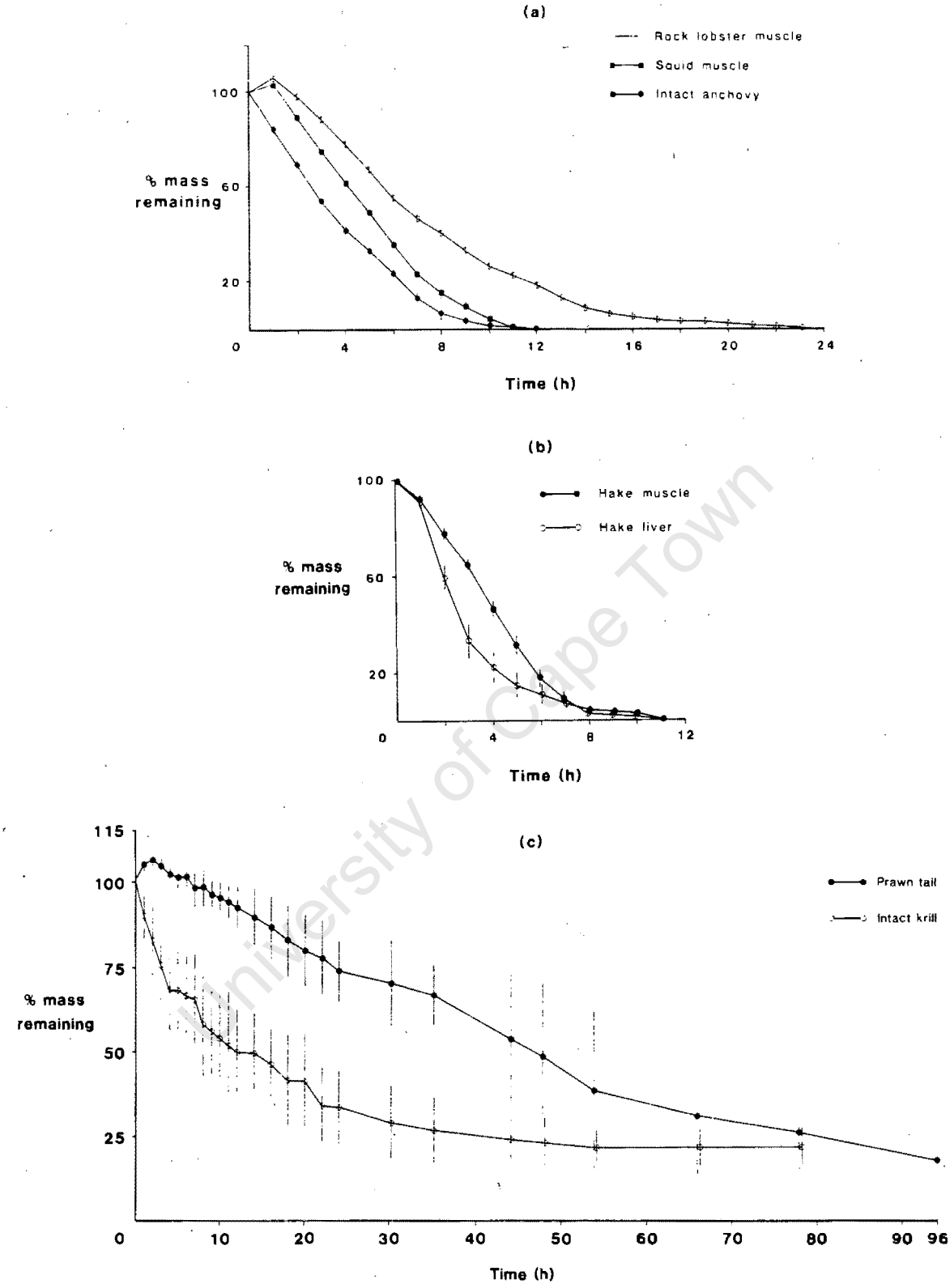

Figure 1.3. Percentages of the mass of different food types remaining at increasing time intervals. Vertical bars represent 1 S.D.

stages of digestion. Prawn and Antarctic Krill were the most indigestible of all food types (Fig. 1.3c). The chitin components of undigested krill and prawn samples constituted 3.2 and 6.4% of total dry mass respectively, whereas corresponding values of samples after *in vitro* digestion were 20.6 and 52.2%.

Times to digestion of 25% of both Rock Lobster muscle and prawn tails were significantly higher than corresponding times for whole anchovy and for hake liver, and hake muscle took less time to digest to this stage than did prawn tails ($H = 45.8211$, $P < 0.001$, $df = 6$; $P < 0.01$ for all pairwise comparisons). The same differences persist amongst times to digestion of 50 and 75% of the samples, with the addition that hake liver lost mass more rapidly than did intact krill ($H = 50.2815$ and 47.3234 for the two cases respectively, $P < 0.001$ and $df = 6$ in both cases; $P < 0.01$ for all pairwise comparisons). Times to complete digestion (100% mass loss) were more rapid for both types of hake tissue than for Rock Lobster muscle ($H = 25.6694$, $P < 0.001$, $df = 4$; $P < 0.01$ for both pairwise comparisons). Because prawn and krill exoskeletons were not digested, it was not possible to calculate times for complete digestion of these prey types.

Mean retention times

Mean retention times (in hours) were 4.78 ± 0.77 for intact anchovy, 5.89 ± 0.73 for squid muscle, 11.73 ± 3.01 and 28.57 ± 3.10 for intact krill and prawn tails respectively, and 8.61 ± 1.44 for Rock Lobster muscle. Among food types, the overall difference was highly significant ($H = 27.7662$, $df = 3$, $P < 0.001$). Values were lower for anchovy than for both krill and prawn ($Y_m/S_m = 3.3971$ and 4.9109 for the two comparisons respectively, $P < 0.01$ in both cases), and mean retention times were significantly lower for squid than for prawn ($Y_m/S_m = 3.3826$, $P < 0.01$).

DISCUSSION

Effects of freezing and agitation on digestion rates

The first experiment indicates that *in vivo* studies using frozen food might yield results that are useful for determination of relative digestibility of different prey, but which are unlikely to be representative of absolute digestion rates of fresh prey by predators in their natural habitat.

The failure of the digestive solution to penetrate the intact crustacean exoskeletons indicates that the simulation of peristalsis was far from perfect, but movement does circulate the enzyme. Agitation such as that described here should be used routinely during *in vitro* digestion experiments.

Differential digestibility of prey: implications for ecological and physiological studies

The resistance of prawn and krill exoskeletons to digestion by pepsin, a broad-spectrum protease, is reflected in the elevated chitin content of the remnants of these foods analyzed after termination of the experiments. Chitinous exoskeletal tissues presumably restrict contact between gastric juices and the soft internal tissues of crustacean prey. Mechanical breakdown of chitin, a complex carbohydrate, would be assisted by the specific enzyme chitinase, which should be looked for in the stomachs of marine predators.

The ranking of prey by *in vitro* digestion rates documented here agrees with published results of earlier experiments on seabirds (Wilson *et al.*, 1985; Jackson and Ryan, 1986). The present study thus highlights potential biases in assessment of the relative importance of certain prey types in seabird diets. Bigg and Fawcett (1985), using different prey species, found squid to be more easily digested than fish both *in vitro* and by Northern Fur Seals *Callorhinus ursinus* (Linnaeus). *In vitro* standards are probably only useful for diet studies where the prey eaten are similar to those used in the experiments.

Although there were no differences in *in vitro* digestion rates of fish and squid, marked differences existed between digestion rates of these prey in Jackass Penguins *Spheniscus demersus* (Wilson *et al.*, 1985) and White-chinned Petrels *Procellaria aequinoctialis* (Jackson and Ryan, 1986). These differences cannot be attributed solely to inherent differences in the digestibility of fish and squid, but rather indicate differences in the digestive abilities of the predators.

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CHAPTER 2

USE OF FIBRE-OPTIC ENDOSCOPES IN STUDIES OF GASTRIC DIGESTION IN CARNIVOROUS VERTEBRATES

With J. Cooper, *Comp. Biochem. Physiol.* 91A:303-308 (1988)

SUMMARY

Two methods of assessing gastric digestion rates of three prey types fed to Sooty Albatrosses *Phoebetria fusca* were compared: removal of stomach contents using a water-flushing stomach pump (a technique used commonly in diet studies), and inspection using a fibre-optic gastroscope (a previously unused technique).

The stomach pump yielded quantitative information, but proved stressful and resulted in incomplete recovery of meals ingested 3 to 6 hr before pumping. Gastric morphology of the animals studied and digestion state of their stomach contents may influence the effectiveness of this technique.

Inspection using the gastroscope yielded qualitative information only but permitted serial inspection of the same individual, and was less stressful than the stomach pump. Times for total evacuation of the stomach were 6 to 12 hr less when estimated using the gastroscope than when using the stomach pump. The specifications of endoscopes relevant to their use by biologists, and previous non-medical biological uses of endoscopes, are given. Potential uses include underwater observations, sampling of digestive juices and stomach linings for enzyme analyses, observations of ingested prey, and assessment of parasite infestation.

INTRODUCTION

Differential digestibility of prey by carnivorous vertebrates warrants investigation, since it can introduce biases in diet studies by exaggeration of the importance of indigestible prey (Bigg and Fawcett, 1985). Various techniques with different merits have been used on fish, birds and mammals to investigate gastric digestion rates. Killing experimental animals at specified intervals after feeding (e.g. Lifjeld, 1983; Wobeser and Galmut, 1984; Bigg and Fawcett, 1985) ensures complete recovery of stomach contents, but is undesirable, particularly if study animals are rare, if they experience slow population growth, or if serial observations of the same individual are necessary. Stomach pumps have been used in studies of gastric digestion rates in fishes (e.g. Seaburg, 1957; Seaburg and Moyle, 1964), seabirds (Wilson *et al.*, 1985; Jackson and Ryan, 1986; Gales, 1987), shorebirds (Charadrii) (Lifjeld, 1983) and fur seals (Bigg and Fawcett, 1985). However, in procellariiform seabirds the efficiency of the water-flushing stomach pump appears to be inversely proportional to stomach fullness (Ryan and Jackson, 1986), limiting its usefulness for digestion studies. Emetics can be used (Montague and Cullen, 1985), but dosages for most vertebrates have not been calculated and, as with stomach pumps, serial observations are impossible. X-ray machines have been used to investigate gastric motility and gastric emptying times in animals fed meals marked with radio-opaque substances (Duke, 1986a; Partridge, 1986). However, in the case of meals that are neither homogeneous nor liquid, the marker may not bind to the prey tissue (Furness and Laugksch, 1983). The passage rate of the marker will therefore not represent the passage rate of digesta.

Fibre-optic instruments are frequently used in medical diagnosis, where observation of body cavities must be made with repeatability and minimum trauma. Such instruments may be useful in studies of gastric digestion, especially on animals where practical, conservation or humane factors preclude the use of other methods.

In this study, we compare stomach pumping with the use of a fibre-optic gastroscope (a type of endoscope) for determining gastric emptying rates in one

species of procellariiform seabird, the Sooty Albatross (*Phoebetria fusca*).

MATERIALS AND METHODS

Laboratory work was carried out at Gough Island (39°21'S, 09°53'W) in October 1986 and October 1987.

Stomach pump

Eight adult Sooty Albatrosses (*Phoebetria fusca*) were captured and fasted for 48 hr before being hand-fed a mixed meal of 120 - 200 g (4 - 8% of the birds' body masses), comprising equal proportions by mass of pilchard (*Sardinops ocellatus*), squid (*Loligo reynaudii*) and prawns (*Penaeus indicus*). Complete prey animals were cut up so that meals comprised approximately the same number of pieces of each prey type. Individual birds' stomachs were then pumped using a water-flushing technique (Wilson, 1984; Ryan and Jackson, 1986) either 3 hr, 6 hr, 9 hr, 12 hr, 18 hr, 24 hr, 36 hr, or 42 hr after feeding. Birds' stomachs were pumped up to five times, until the water emerging from the stomach was clear.

Recovered stomach contents were sorted, separate wet masses for each of the three prey types determined, and digestion state scored visually. The first eight birds were then released, and the experiment repeated using seven freshly-caught birds whose stomachs were pumped at the same time intervals, excluding the 42-hr interval. A third replicate series with seven birds was terminated when one bird died when its stomach was pumped 3 hr after feeding. The bird from the second replicate series whose stomach had been pumped 6 hr after feeding was subsequently found dead after release.

Gastroscope

An Olympus GIF type P fibre-optic gastroscope with an Olympus CLE 4U/4E cold light source was introduced gently into the stomach via the oesophagus to estimate, qualitatively, the rates of digestion of the above three prey species (also cut into pieces of approximately equal mass) fed to Sooty Albatrosses. The birds did

not attempt to regurgitate on introduction of the gastroscope, possibly because their oesphagi naturally accomodate prey items of greater diameter than the gastroscope (7 mm). The tip of the gastroscope was flexible through 180° by controls near the eyepiece, facilitating thorough inspection of the entire proventriculus (forestomach). For each food type six adult birds were captured and fasted for 24 hr, after which no solid faeces were being voided. Each bird was then hand-fed a single meal of 5 - 8% of bird body mass (150 - 210 g). The birds' stomachs were inspected at 6-hr intervals after feeding, until no undigested stomach contents were discernable. The state of digestion of the contents was noted and visually scored according to the following scales:

Pilchard

0 : intact, no apparent digestion

1 : skin partially/wholly digested, heads still intact, not more than 50% of muscle separated from bones

2 : heads reduced to braincases, 50 - 100% of muscle separated from bones, vertebrae disarticulated

3 : only loose vertebrae, scales, eye-lenses, spines or operculae present

4 : prey completely digested, no hard parts remaining

Squid

0 : intact, no apparent digestion

1 : skin shrinking/disintegrating but still attached to muscle, muscle softened, buccal masses intact

2 : skin completely digested but pigment may still remain, muscle tissue thin/pulpy and disintegrating at edges, buccal masses disintegrating

3 : only loose eye-lenses, beaks or pens remaining

4 : prey completely digested

Crustaceans

0 : intact, no apparent digestion

1 : exoskeleton unchanged and retaining pigment, appendages beginning to separate

from body, muscle blocks apparently undigested

2 : exoskeleton still unchanged but muscle inside disintegrating

3 : exoskeleton transparent/ without pigment, may be bile- stained, no muscle inside exoskeleton

4 : prey completely digested

No attempt was made to estimate the quantity of food remaining in the stomach with the gastroscope, since the field of view was too restricted. A minimum of two birds was inspected at each time interval, with individual birds being inspected no more than three times.

RESULTS

Estimated times to total gastric evacuation for albatrosses fed squid and crustacean prey were shorter when the gastroscope was used than when the stomach pump was used (Table 2.1). Visual scores at each time interval for both techniques are given in Table 2.2. Food recovered by pumping the stomachs of Sooty Albatrosses is expressed as a percentage of original wet meal mass in Fig. 2.1.

DISCUSSION

The stomach-pump technique provides quantitative information that use of the gastroscope cannot, but stomach pumping proved stressful (S. Jackson, personal observation) and, in two cases, was fatal for Sooty Albatrosses. Both fatalities almost undoubtedly resulted from the repeated stomach-pumping necessary to empty stomachs packed with food ingested as recently as 3 to 6 hr previously. Significantly, dissection revealed undigested food remaining in the birds' proventriculi.

Gastric morphology may influence the effectiveness of stomach-pumping for the recovery of stomach contents: the water-flushing method was first developed for penguins (Wilson, 1984), whose proventriculi have the long axis in an antero-posterior orientation (McLelland, 1979). The proventriculus of procellariiform birds is displaced to one side of the body cavity, and is laterally curved when full (see

Table 2.1. Times (hr) to total gastric evacuation of three prey types fed to Sooty Albatrosses, estimated using a gastroscope and a water-flushing stomach pump

	Prey type					
	Pilchard		Squid		Prawn	
	flesh	undig. rem.	flesh	undig. rem.	flesh	undig rem
Gastroscope	24	24	24	30	30	36
Stomach pump	24	24	36	36	36	42

undig. rem.: undigested remains or hard parts such as pilchard otoliths and bones, squid pens and beaks, and prawn exoskeletons.

Table 2.2. Visual scores of digestion state (see text) of three prey types fed to Sooty Albatrosses, obtained using a stomach pump (S) and a gastroscope (G). __: no data. Where ranges are given, scores between individual birds varied. Single values are given when scores for all experimental birds were the same.

Time interval (h)	Prey type					
	Pilchard		Squid		Prawn	
	S	G	S	G	S	G
3	1-2	__	1	__	0	__
6	2	1	1-2	0	1	0
9	3	2	2	1	1	__
12	3	2-3	2	2	2	0
18	3	3	2	2	2	2
24	3	3-4	2	2-3	3	2-3
30	3	4	3	4	3	2-3
36	4	4	4	4	3	4
42	__	__	4	__	3	__

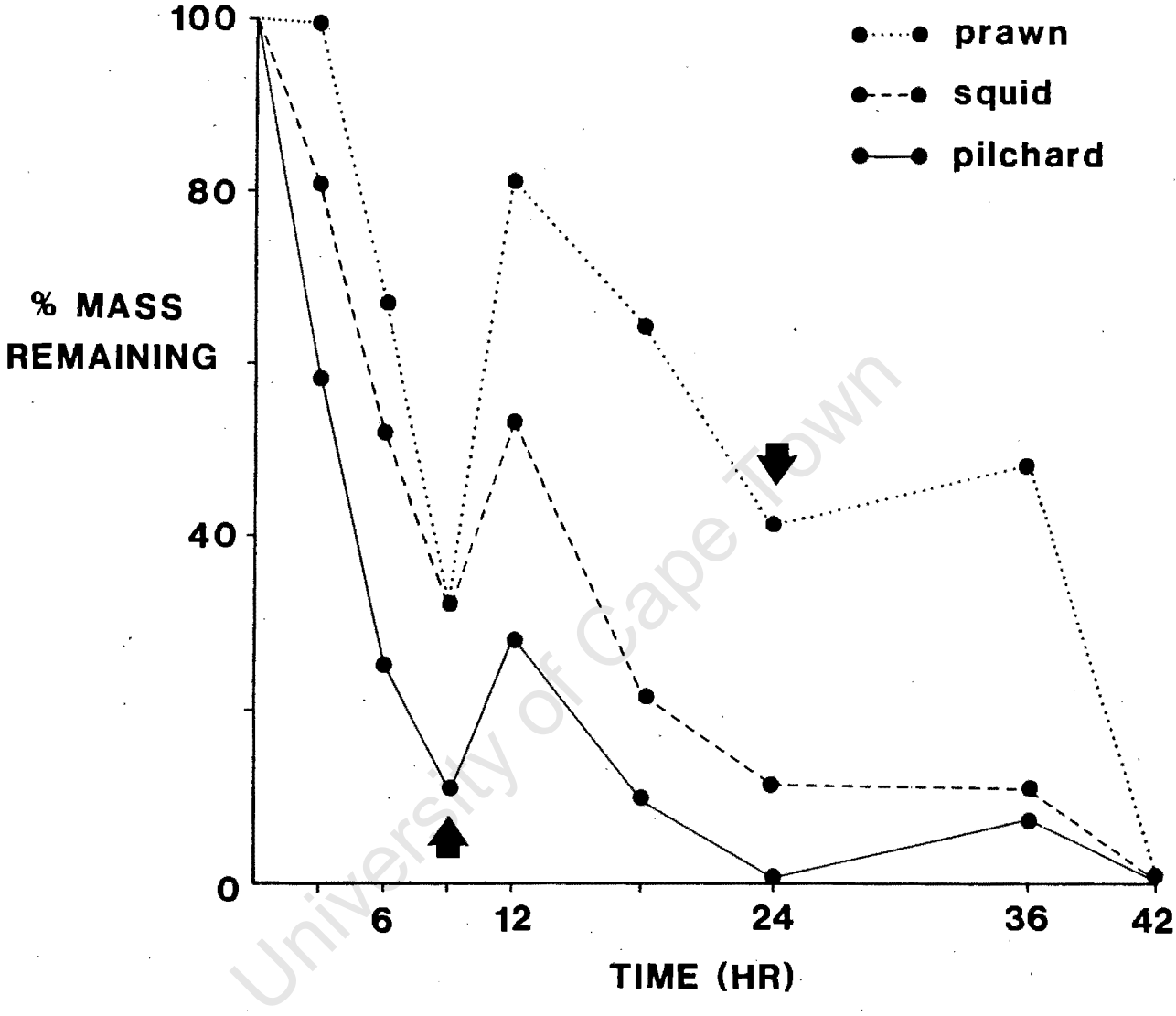

Figure 2.1. Percentage (wet mass) of food recovered by stomach pumping individual Sooty Albatrosses at increasing time intervals after feeding. Arrows indicate possible incomplete recovery of stomach contents.

Chapter 5, Fig. 5.5). This may make the proventriculus more difficult to empty when filled with water and inverted.

Digestion rates of food in some animals may thus be difficult to investigate using the stomach pump, especially shortly after ingestion, when the stomach is still packed. Apparent sharp decreases in mass of stomach contents of the Sooty Albatrosses 9 hr and 24 hr after feeding (Fig. 2.1.) were probably a result of incomplete recovery of the meal rather than of rapid digestion. Stomach pumps are undoubtedly useful tools for diet studies on animals with well- or moderately-digested food in their stomachs (Ryan and Jackson, 1986; Gales, 1987), but great care should be exercised when repeatedly stomach-pumping experimental animals recently fed large meals.

Inspection using gastroscopes is less likely to be stressful than stomach-pumping, especially for procellariiform birds, and the method is preferable for digestion studies because it permits serial inspections of one meal fed to a single animal, which stomach-pumping obviously does not. However, inspection by gastroscope yields only qualitative data that may be subjective. Comparison with data from the stomach pump trials indicates that times to total evacuation of squid and crustaceans from the stomachs of the Sooty Albatrosses appear to have been underestimated by 6 - 12 hr in trials where the gastroscope was used. Small prey remains such as eye-lenses and squid beaks may have been missed during inspection. The ranking of prey according to digestibility was the same for both methods, and thus probably reliable. Photographs taken through a gastroscope may reduce the subjectivity of assessment of digestion state of the prey.

To our knowledge, fibre-optic endoscopes (e.g. gastroscopes, colonoscopes and bronchioscopes) have been little used for non-medical biological purposes, even on such well-studied animals as poultry (Hill, 1983; Duke, 1986b). Olympus endoscopes have flexible insertion tubes with diameters of 7 - 12.6 mm, and lengths up to at least 2 m (White *et al.*, 1978). The tip of the insertion tube may be bent through maximum arcs of 300° in one plane, and 200° in a second plane at right angles to the

first, by controls at the base of the tube near the eyepiece. The maximum field of view is 120°, but most endoscopes offer a field of view of 100°. Depths of fields are 2.3/10 - 100 mm, with either a fixed or adjustable focus. The lens at the tip of the endoscope can be flushed clean with water pumped through the tube, which also has a channel with diameters of 2 - 3.7 mm through which samples of stomach juices can be withdrawn by suction. Biopsy instruments can be operated through these channels. Single lens reflex, cinematograph and video cameras can be fitted to the eyepiece of the endoscope. The light source uses 12 V d.c. (and therefore can be powered by a vehicle battery), and the smallest in size is approximately 140 x 350 x 450 mm, and weighs 14 kg.

Fibre-optic endoscopes have been used to compare gastric digestion rates in Sooty Albatrosses and Rockhopper Penguins (*Eudyptes chrysocome*) (this paper; S. Jackson, unpublished data), and to study and photograph burrow-, hole- and crevice-living animals (e.g. White *et al.*, 1978; Anon., 1987). In a recent diet study an endoscope was used to ensure that Weddell Seals (*Leptonychotes weddelli*) with empty stomachs were not killed unneccessarily (N.T.W. Klages, personal communication). Other possible non-medical biological uses of endoscopes include underwater observations, sampling of digestive juices and stomach linings for enzyme analyses, observations of ingested prey and assessment of parasite infestation.

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CHAPTER 3

GUT SIZE AND PASSAGE RATES OF SOLID DIGESTA IN SEABIRDS

SUMMARY

Gastro-intestinal passage rates of solid digesta were measured for the Cape Gannet *Morus capensis*, Sooty Albatross *Phoebetria fusca*, and Rockhopper *Eudyptes chrysocome*, Gentoo *Pygoscelis papua* and King *Aptenodytes patagonicus* penguins fed fish, squid and crustaceans of known *in vitro* digestibility. Gut retention times of the three food types show the same ranking as estimated gastric evacuation times for these seabirds. The mean retention times reported here are probably the first such data for seabirds eating vertebrate prey.

Gut lengths and volumes of the same five seabirds were measured, and mean retention time was found to be significantly correlated with hindgut length. Hindgut length, volume, and planar surface area all scaled significantly with body mass. The exponent of body mass differed between flying seabirds and penguins, significantly so in the case of hindgut area. Mean retention time is thus indirectly influenced by body mass. Gut passage rates are not invariably shorter in flying species than in penguins. Mean retention times are significantly correlated with foraging trip duration for breeding adults (in days, taken from the literature).

Prey types prevalent in the natural diet of each species may pass through the gut of that species more rapidly than do less favoured prey. Despite its relative indigestibility *in vitro*, squid is excreted faster than is fish by the four seabirds that breed at islands in the Southern Ocean, where squid is abundant. The Cape Gannet excretes pilchard, its major natural prey item, more rapidly than it does squid.

INTRODUCTION

The availability of energy limits breeding success in seabirds (Lack, 1968), and growth rates of juvenile birds may be influenced by the rate at which they are able to process food (Ricklefs, 1968). Ricklefs (1983) has discussed the constraints on parent birds transporting energy from feeding grounds to their chicks, concluding that transportation of food, rather than foraging ability, may limit seabirds' reproductive output. Digestive parameters such as gut passage time influence seabirds' abilities to transport food by affecting both the rate of energy gain by the parent bird, and the rate of delivery of food to chicks during the breeding season. Interspecific differences in digestion rates and gastro-intestinal passage of digesta in seabirds may thus reflect adaptations to maximise digestive efficiency within the constraints imposed by different lifestyles.

In addition to these constraints, gastric emptying rate, hence feeding frequency, is influenced by prey digestibility. An early study on Cape Gannets, Cape Cormorants (*Phalacrocorax capensis*) and Jackass Penguins (*Sphensicus demersus*) (Davies, 1956) indicates that complete digestion of fish takes between two and six hours, whereas the White-breasted Cormorant (*P. carbo sinensis*) may take up to 15 hours to digest a 30-cm fish (van Dobben, 1952). Published studies comparing differential digestion rates of prey within seabird species (Wilson *et al.*, 1985; Jackson and Ryan, 1986) are few, as are studies of gastro-intestinal transit of similar foods between species (Laugksch and Duffy, 1986). Such studies are needed both to highlight potential biases in avian diet studies (Hartley, 1948; Lifjeld, 1983; Wilson *et al.*, 1985), and to improve our understanding of digestive adaptations in seabirds.

This study investigates the influences of foraging method and diet on gut passage rate in five seabird species with different natural diets and foraging methods: a sulid (the Cape Gannet, *Morus capensis*), an albatross (the Sooty Albatross, *Phoebetria fusca*), and three penguins (the Rockhoppper, *Eudyptes chrysocome*; Gentoo, *Pygoscelis papua*; and King, *Aptenodytes patagonicus* penguins). These species were fed fish, squid and crustaceans of known *in vitro* digestibility (see

Chapter 1). Interspecific comparisons are used in combination with allometry, to test the validity of three predictions arising from the premise that seabird digestive processes have adaptive significance. Firstly, penguins should exhibit slower gut passage rates than flying species, because flightless birds are less subject to weight-related energetic constraints while foraging. For the same reason, penguin guts should be larger in relation to body size. Thirdly, adaptation to specialized diets may be reflected in faster gut passage times for frequently eaten prey than for less important food types.

MATERIALS AND METHODS

Feeding experiments

Experimental work on Sooty Albatrosses and non-moulting (pre-breeding) northern Rockhopper Penguins was carried out at Gough Island (39°21'S, 9°53'W) in October 1986 and October 1987. King, Gentoo and post-moulting Rockhopper Penguins were used in experiments at Marion Island (46°54'S, 37°45'E) in April and September 1987. Cape Gannets were caught at Malgas Island (33°03'S, 17°55'E) in Saldanha Bay, and taken to the University of Cape Town for the duration of the experiments.

Three food types were fed to the birds: pilchard (*Sardinops ocellatus*), squid (*Loligo vulgaris reynaudii*), and prawn (*Penaeus indicus*). Prawns were used as a substitute for the euphausiid crustaceans naturally eaten by the seabirds, because euphausiids were not available at the time of the feeding experiments. Food was stored frozen and thawed immediately before use. Previously-frozen food is more rapidly digested *in vitro* than is fresh food (Chapter 1), but the ranking of digestibilities of different food types are consistent for both treatments. The predictions addressed in this paper relate to relative digestibilities of different prey, and I have assumed that the consistencies of all food types are affected to the same degree by freezing.

Time was limiting, and the birds could not be induced to feed *ad libitum*, so

individuals were force-fed single meals of wet mass adjusted to fall within the range of meal sizes eaten by their conspecifics in the field. These data were obtained from Navarro and Adams (ms) for Cape Gannets, Croxall *et al.* (1985) for Rockhopper Penguins, Adams and Wilson (1987) for Gentoo Penguins, and Adams and Klages (1987) for King Penguins. In the case of the Sooty Albatross, there are no such published data, so the size of meals fed by adult Light-mantled Sooty Albatrosses *P. palpebrata* to their chicks at South Georgia (Thomas, 1982), was used as a reference. The two *Phoebetria* species are of similar body mass (Berruti, 1977).

Five to six adult birds were used in feeding trials with every bird species and with each prey type. Individual birds were held captive only for the duration of a single feeding trial. The birds were housed in a laboratory in cylindrical plastic barrels with open tops and galvanized metal mesh floors (mesh size 25 x 25 mm). The cages were suspended over fibreglass or plastic funnels emptying into plastic jars. The laboratory was kept at ambient temperature and was subject to the natural diel light-dark cycle. Before the feeding trials commenced, the birds were fasted for 48 - 72 hours, until their faeces were bile-green or black in colour and appeared to be of secretory origin rather than undigested dietary residue. After fasting, each bird was fed a single meal (see above), and its faeces collected at two-hourly intervals until no solid matter was being voided, whereupon the bird was released. Faeces were collected from the sides of the cage and funnel and from the cage floor by rinsing with a fine stream of water directed from above the open top of the cage using a squeeze bottle. This method minimized disturbance of the birds, because it did not necessitate moving the cages. Collection and weighing of residue from the cages after one feeding trial showed that 1.0 - 1.7% of the total dry mass of faeces excreted escaped collection. The cages and funnels were cleaned of all faeces between each feeding trial.

Faecal samples for each bird for each time interval were transferred to plastic bags, frozen at -20°C together with the rinsing water, and shipped to the University of Cape Town. Here they were thawed, transferred to pre-weighed aluminium foil

dishes, and dried to constant mass at 45°C before weighing. Samples for all time intervals were then pooled for each bird for further analyses (see Chapter 4).

The influence of meal size on mean retention time was assessed by feeding pilchard meals of four different approximate sizes (350 g, 750 g, 1000 g and two meals, each of approximately 300 g, fed ten hours apart) to four groups of King Penguins. The experimental procedure for these feeding trials was identical to that described above.

The above technique was compared with a dyed-meal method adapted from Duffy *et al.* (1985). Four adult Sooty Albatrosses were fed a meal of pilchard that had been marked with 0.5 ml of a 10% suspension of carmine dye in distilled water, poured into five gelatin capsules that were embedded in the muscle of the prey. For logistic reasons, it was not possible to maintain live fish and inject them with carmine as in the study by Duffy *et al.* (1985).

Faecal samples from individual birds for each time interval were collected using the methods described above, but after drying and weighing each sample was rehydrated and homogenized by forcing it through a seive with mesh size 0.5 mm. For comparative purposes, samples were processed using the procedure described by Duffy *et al.* (1985). The homogenized solution was made up to 300 ml with distilled water, and the solids allowed to settle out for 24 hours before reading the optical density of the solution on a Beckman DU-40 spectrophotometer at 520 nm. Carmine concentrations for each sample were calculated using a calibration curve drawn up from readings for carmine solutions of known concentrations. The faeces of one of the birds fed unmarked meals were analyzed in the same way.

The experiment was repeated using different birds fed carmine-marked squid and prawn meals.

Passage rates of solid digesta in Sooty Albatrosses and Rockhopper Penguins were compared with aqueous marker excretion rates for the same species The aqueous marker used was [^{3}H] polyethylene glycol, and the experimental procedure is described in Chapter 5.

Gastric emptying times

Times to complete gastric evacuation were estimated by serial inspection of the stomachs of Cape Gannets, Sooty Albatrosses, and Rockhopper and King Penguins fed pilchard, squid and prawn, using a fiber-optic gastroscope (see Chapter 2). Statistical comparisons between species were not attempted because of the qualitative nature of this information (see Chapter 2).

Gut measurements

Four adult King and four adult Rockhopper penguins at Marion Island, and five adult Cape Gannets at Malgas Island, Saldanha Bay, were used for gut measurements. Measurements from one Rockhopper Penguin and one Sooty Albatross (Chapter 5) were incorporated into the data set, and values for eight other seabird species (Figs 3.2, 3.3 and 3.4) were taken from R.C. Laugksch (unpubl. data). All measurements were made in the same manner. Birds were killed under permit by intravenous injection of "Euthanaze" (a stable solution containing 200 mg sodium pentobarbitone per ml, Centaur Laboratories, Johannesburg, South Africa). Carcasses were frozen intact and shipped to the University of Cape Town, where they were thawed and the gastro-intestinal tracts dissected out. The lengths of the foregut (oesophagus and stomach) and hindgut (small and large intestines, and rectum) were measured to the nearest mm using a 50-cm ruler. Twists and loops in the intestines were straightened out, without stretching the guts. All mesenteries and fat were then removed, the gut contents squeezed out, and the fore- and hindguts weighed to the nearest 0.1 g. Hindgut planar surface area was estimated as the product of gut length and the mean width of four opened cross-sections taken at points equidistant along the hindgut. Hindgut diameter was calculated as the mean gut width/pi, and hindgut volume as the product of cross-sectional area ($pi.r^2$) and length.

Statistics and data analysis

Excretion curves for each bird species and each food type were plotted as the

mean cumulative percentages of faeces excreted at increasing time intervals since feeding, with grams dry mass of faeces as the original units of measurement. Mean retention times of digesta in the birds' guts were calculated for each species for each food type, using the formula:

$$t = \sum_{i=1}^{n} m_i t_i \Big/ \sum_{i=1}^{n} m_i$$

where t is mean retention time of digesta in the gut, and m_i is the amount of faeces (g dry mass) excreted at time interval t_i after feeding (Warner, 1981). Mean retention time calculated for a particular time interval is independent of faecal collections subsequent to that time interval, unlike the percentages of faeces excreted at each time interval, which are influenced by the differing times to completion of individual feeding trials. Mean retention times were thus the basis for all interspecific comparisons. The frequency and total number of faecal collections influence values of mean retention time calculated using Warner's (1981) formula. For the present study, faeces were collected at the same time intervals for all feeding trials. All inter- and intraspecific comparisons are restricted to corresponding intervals. Mean retention times calculated at each time interval are an integrated expression of the speed of excretion up to that interval, and statistical comparisons between these values yield insight into progressive stages of digestion. Correlations between foraging parameters or gut size and mean retention time were calculated using the maximum time interval for which data are available for all species (48 hours).

Non-parametric statistical tests were used for all inter- and intraspecific pairwise comparisons. The Kruskal-Wallis single factor analysis of variance confirmed significant between-group variances, whereafter significantly different species pairs or pairs of food types were isolated with an *a priori* test that uses rank sums (Dunn, 1964). Two-tailed Wilcoxon U-tests were used for simple pairwise comparisons.

A sampled randomization test (Sokal and Rohlf, 1969) was used to test for

significant differences between mean retention times of the three food types fed to Sooty Albatrosses, estimated using the carmine and gravimetric experimental methods. A modified form of the student's t-test (Zar, 1974) was used to detect differences between regression slopes and intercepts.

RESULTS

Table 3.1 shows mean body masses and mean wet meal masses for each species used in the passage rate feeding trials.

Mean retention times

All the differences discussed below are statistically significant at levels denoted by asterisks in Tables 3.2, 3.3 and 3.4. Values for the Kruskal-Wallis H-statistic and for Y_m/S_m for each pairwise comparison, are given in Appendices 3.1 to 3.3. Amongst birds fed pilchard, Cape Gannets and Gentoo Penguins exhibited consistently lower mean retention times than did King Penguins (Table 3.2). Furthermore, mean retention times for the gannets were lower than those for Sooty Albatrosses and Rockhopper Penguins at, respectively, the 18- and 24-hour time intervals. At the 42-hour time interval, Gentoo Penguins exhibited lower mean retention times than did both Rockhopper Penguins and Sooty Albatrosses. Amongst birds fed squid, gannet mean retention times were initially lower than those for Sooty Albatrosses (Table 3.3). Cape Gannets and Gentoo Penguins retained squid for shorter periods than did King Penguins. Twelve hours after feeding, mean retention times of squid were less in Gentoo Penguins than in the Sooty Albatross. After 48 hours, mean retention times in Sooty Albatrosses were lower than those for King Penguins. Cape Gannets fed prawn also exhibited significantly shorter mean retention times than did King Penguins fed this food (Table 3.4). After 18 hours, Cape Gannets retained prawn for less time than did Rockhopper Penguins. After 48 hours, Sooty Albatrosses retained this food type for shorter periods than did King Penguins.

In most within-species comparisons of the different food types, prawn was

Table 3.1. Mean wet meal masses (g) and body masses (kg) for birds used in the feeding experiments. Standard deviations in parentheses. BM: body mass. MM: meal mass. N = 6 in all cases except the feeding trial in which Sooty Albatrosses were fed pilchard, and that in which King Penguins were fed squid. In these two instances, N = 5.

Species		Food type		
		Pilchard	Squid	Prawn
Cape Gannet	BM	2.4 (0.1)	2.4 (0.2)	2.4 (0.2)
	MM	222.6 (6.6)	236.7 (3.8)	221.4 (6.7)
Sooty Albatross	BM	2.5 (0.3)	2.4 (0.2)	2.4 (0.2)
	MM	346.3 (32.1)	357.7 (61.1)	354.1 (2.3)
Rock-hopper Penguin	BM	2.5 (0.2)	2.5 (0.2)	2.5 (0.4)
	MM	153.9 (20.1)	159.7 (3.7)	138.7 (7.1)
Gentoo Penguin	BM	5.8 (0.5)	6.3 (0.5)	6.0 (0.6)
	MM	426.4 (20.0)	410.8 (7.2)	411.3 (1.7)
King Penguin	BM	11.6 (0.5)	11.1 (1.1)	11.0 (1.0)
	MM	738.4 (11.6)	747.9 (11.2)	626.9 (10.2)

Table 3.2. Mean retention times (h) of pilchard by Cape Gannets, Sooty Albatrosses, and Rockhopper, Gentoo and King Penguins. Standard deviations in parentheses. Significantly different species pairs within each time interval indicated by asterisks. *: $P < 0.05$, **: $P < 0.01$. Figures in parentheses below species names = sample sizes. n.d.: no data

Time interval (h)	Species 1. Cape Gannet (n = 5)	2. Sooty Albatross (n = 7)	3. Rockhopper Penguin (n = 6)	4. Gentoo Penguin (n = 6)	5. King Penguin (n = 6)	Significant differences between species pairs
6h	4.04 (0.17)	4.14 (0.37)	4.35 (0.23)	4.45 (0.25)	4.86 (0.29)	1 *v* 5*
12h	5.54 (0.47)	7.26 (0.71)	7.22 (0.41)	6.37 (0.50)	8.09 (0.27)	1 *v* 5** 4 *v* 5**
18h	6.29 (0.53)	9.89 (1.09)	n.d.	8.05 (0.68)	11.24 (0.32)	1 *v* 2*; 1 *v* 5** 4 *v* 5
24h	7.98 (1.04)	11.92 (1.23)	12.44 (1.12)	9.24 (1.21)	13.70 (0.60)	1 *v* 3*; 1 *v* 5** 4 *v* 5**
30h	9.21 (1.09)	12.96 (1.14)	13.59 (1.33)	10.02 (1.16)	15.67 (0.90)	1 *v* 5** 4 *v* 5**
36h	n.d.	14.44 (1.64)	14.34 (1.39)	11.22 (1.22)	17.48 (1.22)	4 *v* 5**
42h	n.d.	15.65 (1.98)	15.21 (1.50)	11.87 (1.27)	n.d.	1 *v* 4* 3 *v* 4*
48h	n.d.	16.69 (2.25)	16.31 (1.78)	13.74 (1.64)	21.45 (2.63)	4 *v* 5**
54h	n.d.	n.d.	n.d.	15.41 (1.32)	22.60 (2.91)	4 *v* 5**

Table 3.3. Mean retention times (h) of squid by Cape Gannets, Sooty Albatrosses, and Rockhopper, Gentoo and King Penguins. Notation as for Table 3.2.

Time interval (h)	Species 1. Cape Gannet (n = 5)	2. Sooty Albatross (n = 7)	3. Rockhopper Penguin (n = 6)	4. Gentoo Penguin (n = 6)	5. King Penguin (n = 5)	Significant differences between species pairs
6h	3.99 (0.33)	4.82 (0.39)	4.73 (0.38)	4.31 (0.32)	4.46 (0.29)	1 *v* 2*
12h	5.43 (0.55)	7.68 (0.34)	6.93 (0.51)	5.84 (0.50)	7.95 (7.94)	1 *v* 2**; 1 *v* 4** 1 *v* 5**; 2 *v* 4* 4 *v* 5**
18h	6.85 (0.90)	9.35 (0.47)	8.82 (0.58)	7.11 (0.93)	10.96 (0.80)	1 *v* 5** 4 *v* 5**
24h	8.59 (1.24)	10.37 (0.71)	9.94 (0.57)	8.59 (0.62)	12.92 (0.48)	1 *v* 5** 4 *v* 5**
30h	10.41 (1.25)	11.21 (0.61)	10.73 (0.70)	9.58 (0.70)	14.21 (1.05)	4 *v* 5**
36h	n.d.	12.00 (0.55)	11.56 (0.89)	10.75 (1.07)	15.25 (1.19)	4 *v* 5**
42h	n.d.	12.60 (0.52)	12.78 (1.50)	12.25 (1.16)	16.70 (1.53)	4 *v* 5**
48h	n.d.	13.46 (0.55)	14.03 (1.51)	13.94 (1.27)	17.93 (1.97)	2 *v* 5*
54h	n.d.	n.d.	n.d.	15.40 (1.15)	19.45 (2.08)	4 *v* 5**

Table 3.4. Mean retention times (hours) of prawn by Cape Gannets, Sooty Albatrosses, and Rockhopper, Gentoo and King Penguins. Notation as for Table 3.2.

Time interval (h)	Species 1. Cape Gannet (n = 6)	2. Sooty Albatross (n = 6)	3. Rockhopper Penguin (n = 6)	4. Gentoo Penguin (n = 5)	5. King Penguin (n = 5)	Significant differences between species pairs
6h	3.67 (0.30)	4.82 (0.25)	n.d.	4.66 (0.78)	3.83 (2.15)	
12h	5.79 (0.36)	7.68 (0.65)	7.47 (0.83)	7.72 (0.86)	8.50 (1.11)	1 *v* 5*
18h	8.08 (0.46)	10.47 (0.60)	10.85 (0.58)	9.69 (1.44)	11.53 (0.93)	1 *v* 3*; 1 *v* 5** 4 *v* 5**
24h	10.12 (0.37)	12.96 (1.41)	13.63 (1.00)	12.18 (1.67)	14.48 (1.05)	1 *v* 5*
30h	11.68 (0.31)	n.d.	15.42 (1.12)	14.15 (1.90)	16.64 (1.06)	1 *v* 5*
36h	12.75 (0.37)	15.84 (2.82)	16.69 (1.41)	14.73 (2.14)	18.60 (1.53)	1 *v* 5*
42h	n.d.	16.68 (3.28)	18.10 (1.22)	16.33 2.16)	21.16 (2.49)	
48h	16.18 (0.99)	17.33 (3.76)	20.20 (1.27)	18.07 (1.70)	22.70 (2.78)	
54h	18.44 (1.02)	n.d.	21.61 (1.45)	19.49 (1.46)	24.52 (3.08)	1 *v* 5*
60h	n.d.	18.16 (3.74)	22.93 (1.24)	n.d.	25.96 (3.57)	2 *v* 5*

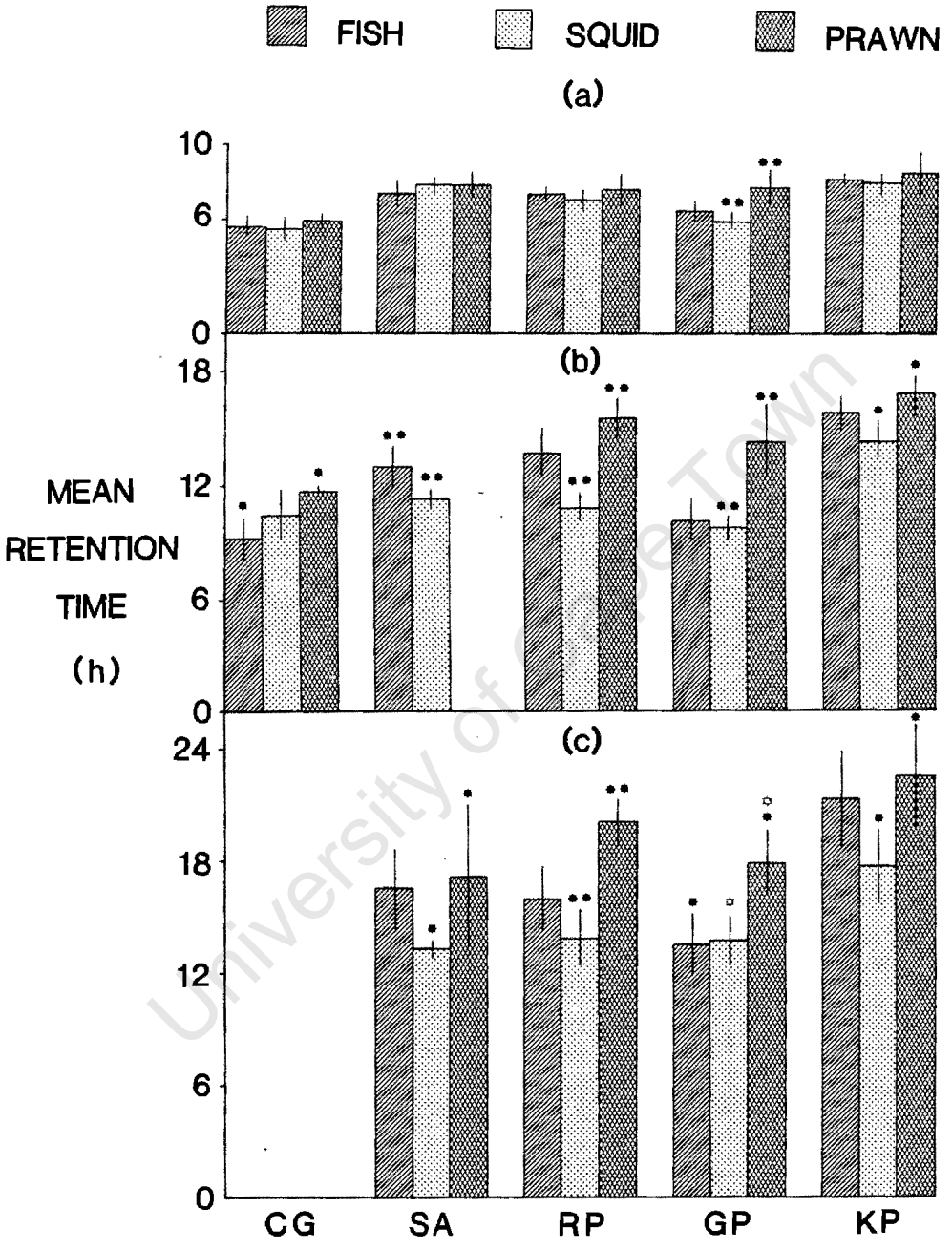

Figure 3.1. Mean retention times (hours) of the three food types grouped according to seabird species. CG: Cape Gannet; SA: Sooty Albatross; RP: Rockhopper Penguin; GP: Gentoo Penguin; KP: King Penguin. Vertical bars denote 1 standard deviation. *: $P < 0.05$ **: $P < 0.01$. Only mean retention times calculated up to 18 hours (a), 30 hours (b) and 48 hours (c) after feeding, are depicted.

retained for significantly longer periods than were either pilchard or squid (Fig. 3.1). Amongst Cape Gannets, mean retention times calculated 30 hours after feeding were shorter in birds fed pilchard than in birds fed prawns ($P < 0.05$, Fig. 3.1). In contrast, squid was retained for the shortest times of all foods by the other four seabird species (Fig. 3.1). Gentoo Penguins consistently retained pilchard for significantly shorter times than they did prawn.

Allometry of the gut

Independent of body mass, Cape Gannets had the shortest hindguts, followed by the Sooty Albatross, and Gentoo, Rockhopper and King Penguins in order of increasing gut length (Table 3.5). Hindgut mass was not directly proportional to length, because gannet hindguts were both shorter and heavier than that of the Sooty Albatross, and the hindgut of the Gentoo Penguin was heavier than those of the Rockhopper Penguins measured. Rockhopper Penguins had long, narrow hindguts whereas that of the Gentoo Penguin was wide and short.

For 13 seabird species, hindgut length scaled positively with $(\text{body mass})^{0.65}$ (Fig. 3.2). The correlation coefficient for these two parameters for penguins alone was well below significance levels, but hindgut length scaled significantly with $(\text{body mass})^{0.37}$ for flying species (see Fig. 3.2 for regression equations and correlation coefficients). There was no significant difference between the slopes of the two regressions shown in Fig. 3.2. Hindgut volume scaled positively with $(\text{body mass})^{1.09}$ for all species, and with $(\text{body mass})^{0.81}$ for flying species only (see Fig. 3.3 for correlation coefficients and equations). Again, the slopes and y-intercepts of the two regressions did not differ significantly. For penguins alone, hindgut volume scaled with $(\text{body mass})^{1.27}$, but the relationship was not statistically significant.

Hindgut surface area scaled with $(\text{body mass})^{0.58}$ among flying species, and with $(\text{body mass})^{0.66}$ for penguins (Fig. 3.4). Both the slopes and the y-intercepts of these two regressions differed significantly ($t_9 = 10.940$, $P < 0.001$; and $t_{10} = 3.636$, $P < 0.005$, respectively).

Mean retention time of all foods was not significantly correlated with body

Table 3.5. Gut measurements of the Cape Gannet, Sooty Albatross and Rockhopper, Gentoo and King Penguins. Figures in parentheses after species names = sample sizes. F = foregut, H = hindgut, T = total (foregut plus hindgut). Figures in parentheses after measurements = standard deviations. n.d. = no data.

Measurement		Species				
		Cape Gannet (6)	Sooty Albatross (1)	Rockhopper Penguin (5)	Gentoo Penguin (1)	King Penguin (4)
Body mass (kg)		2.39	2.15	3.46	6.05	12.05
Gut mass (g)	F	48.93 (3.12)	18.80	29.70	n.d.	n.d.
	H	21.45 (2.38)	17.80	48.28 (6.47)	120.04	187.42 (50.15)
	T	70.48 (3.47)	36.60	77.98	—	—
Gut length (cm)	F	44.5 (1.2)	11.8	13.0	n.d.	n.d.
	H	127.8 (5.8)	151.9	523.5 (38.7)	314	672.3 (54.05)
	T	172.3 (6.3)	163.7	536.5	—	—
Hindgut diameter (mm)		5.07	5.41	4.23	13.81	8.91
Hindgut volume (cm^3)		25.86	34.01	75.046	470.473	414.31
Planar surface	F	238.3 (4.1)	56.3	126.3	—	—
area (cm^2)	H	203.8 (17.1)	254.8	702.6 (193.9)	1362.5	1870.9 (67.46)
	T	442.0 (16.8)	311.1	828.9	—	—

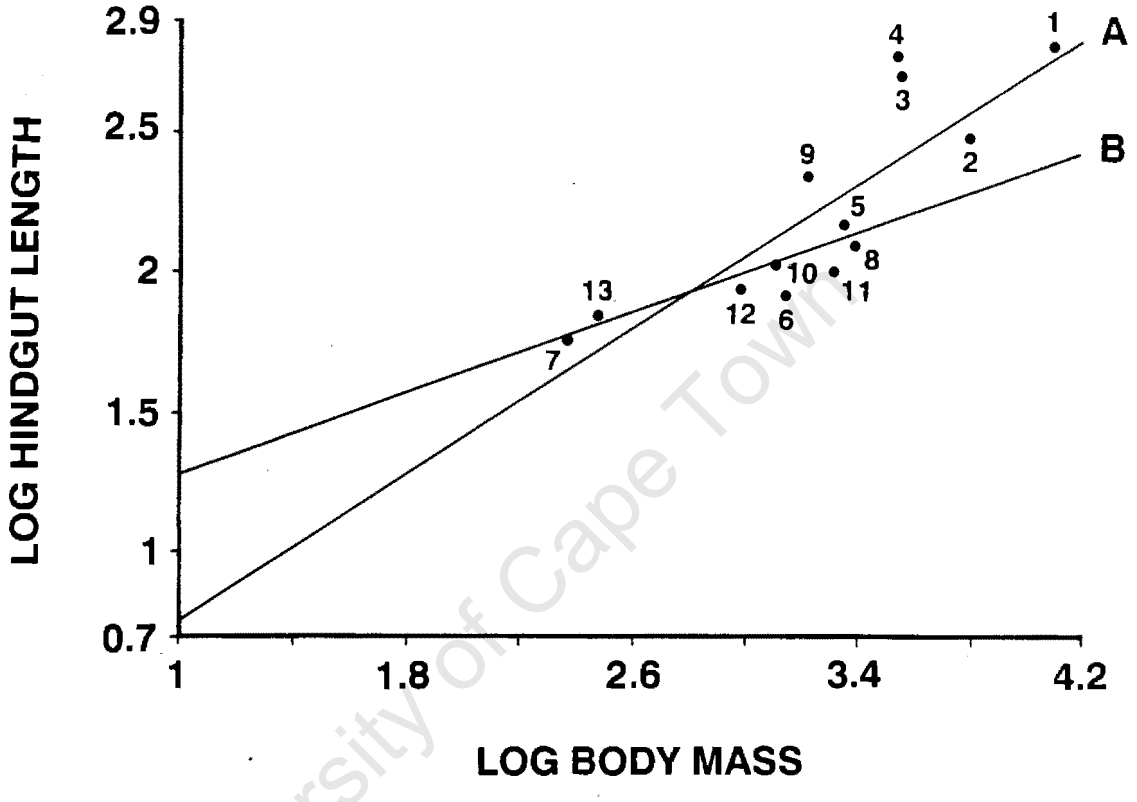

Figure 3.2. Scaling of hindgut length (y, cm) against body mass (x, kg) for thirteen seabird species.

A: all species: $\log y = 2.07 + 0.65 \log x$, $r_{11} = 0.84$, $P < 0.001$.

B: flying species only: $\log y = 2.01 + 0.37 \log x$, $r_7 = 0.76$, $P < 0.02$,

where y = hindgut length and x = body mass.

Species codes: (sample sizes in parentheses after species names)

1: King Penguin *Aptenodytes patagonicus* (4) 2: Gentoo Penguin *Pygoscelis papua* (1) 3. Rockhopper Penguin *Eudyptes chrysocome* (5) 4: Jackass Penguin *Spheniscus demersus* (3) 5: Sooty Albatross *Phoebetria fusca* (1) 6: White-chinned Petrel *Procellaria aequinoctialis* (1) 7: Little Shearwater *Puffinus assimilis* (1) 8: Cape Gannet *Morus capensis* (5) 9: White-breasted Cormorant *Phalacrocorax carbo* (1) 10: Cape Cormorant *Phalacrocorax capensis* (3) 11: Subantarctic Skua *Catharacta antarctica* (1) 12: Kelp Gull *Larus dominicanus* (1) 13: Hartlaub's Gull *Larus hartlaubii* (1).

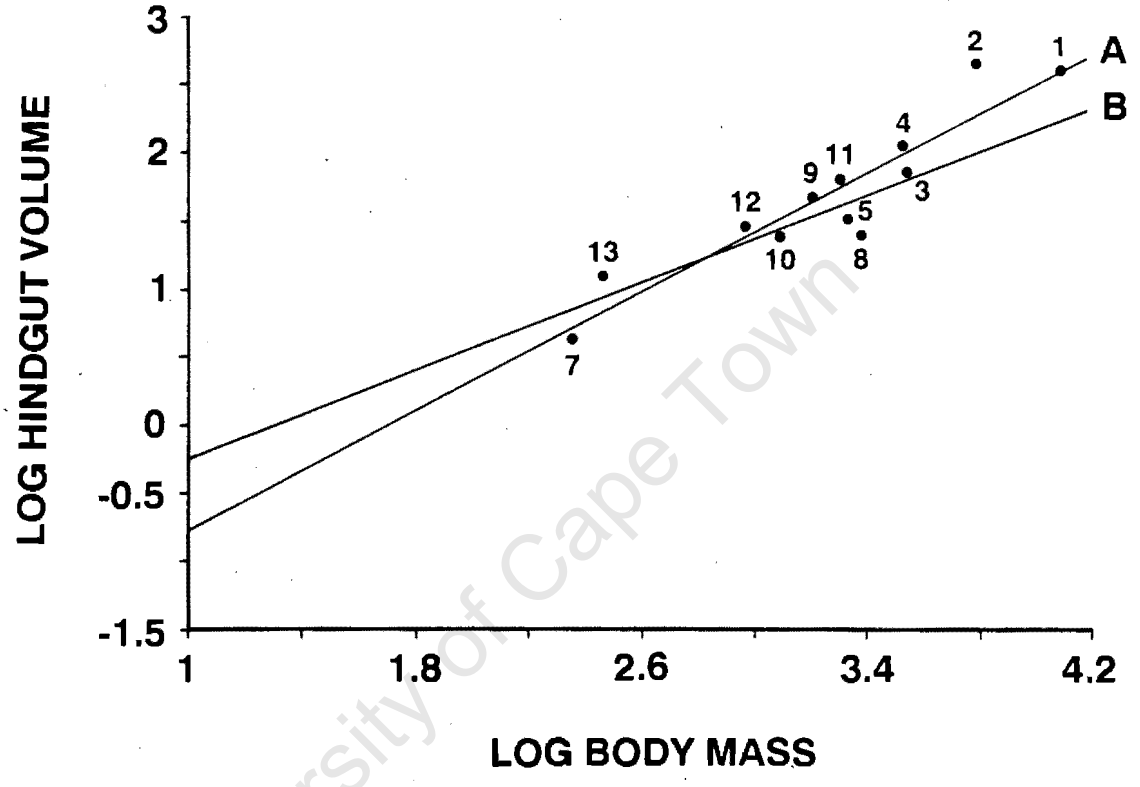

Figure 3.3. Scaling of hindgut volume (y, cm^3) to body mass (x, kg) for twelve seabird species. Species codes as for Fig. 3.2.

A: All species: $\log y = 1.41 + 1.09 \log x$, $r_{10} = 0.93$, $P < 0.001$.

B: Flying species only: $\log y = 1.37 + 0.81 \log x$, $r_6 = 0.87$, $P \leq 0.005$.

(Penguins only: $\log y = 1.38 + 1.27 \log x$, $r_2 = 0.84$, $0.2 > P > 0.1$, NS).

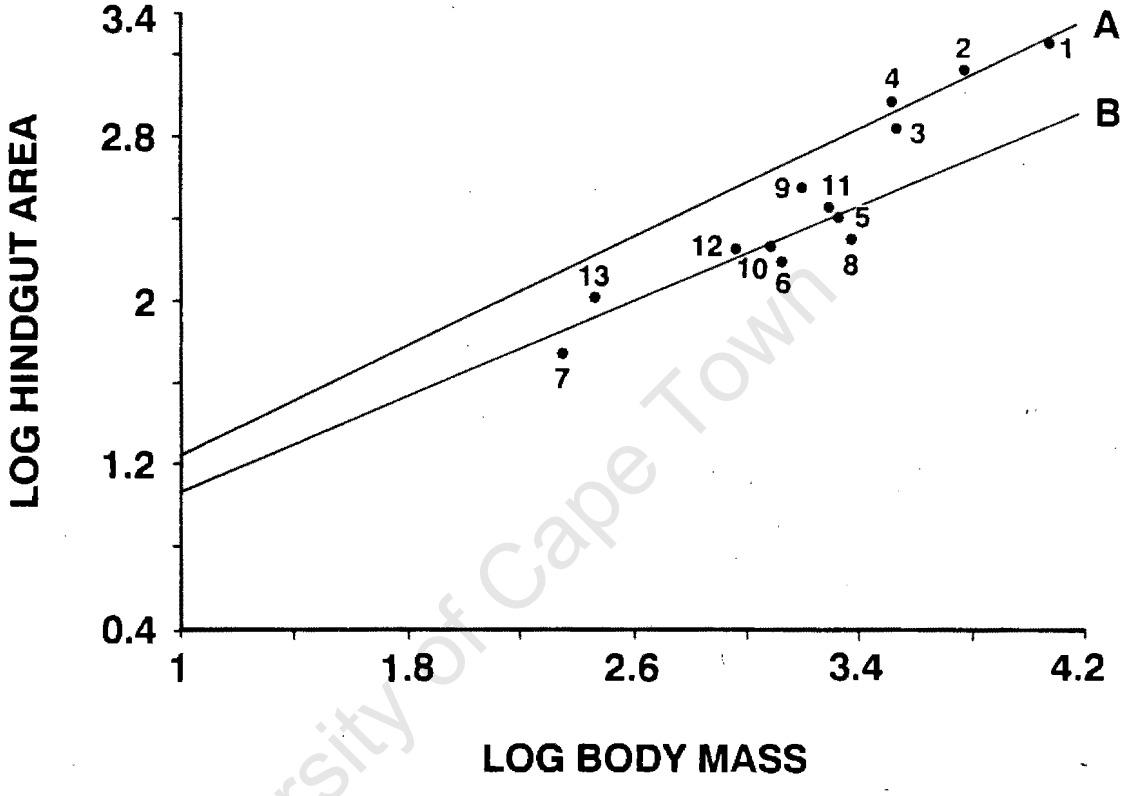

Figure 3.4. Scaling of hindgut area (y, cm^2) to body mass (x, kg) for thirteen seabird species. Species codes as for Fig. 3.2.

A: Penguins only: $\log y = 0.58 + 0.66 \log x$, $r_2 = 0.95$, $P \leq 0.05$.

B: Flying species: $\log y = 0.49 + 0.58 \log x$, $r_7 = 0.88$, $P < 0.002$.

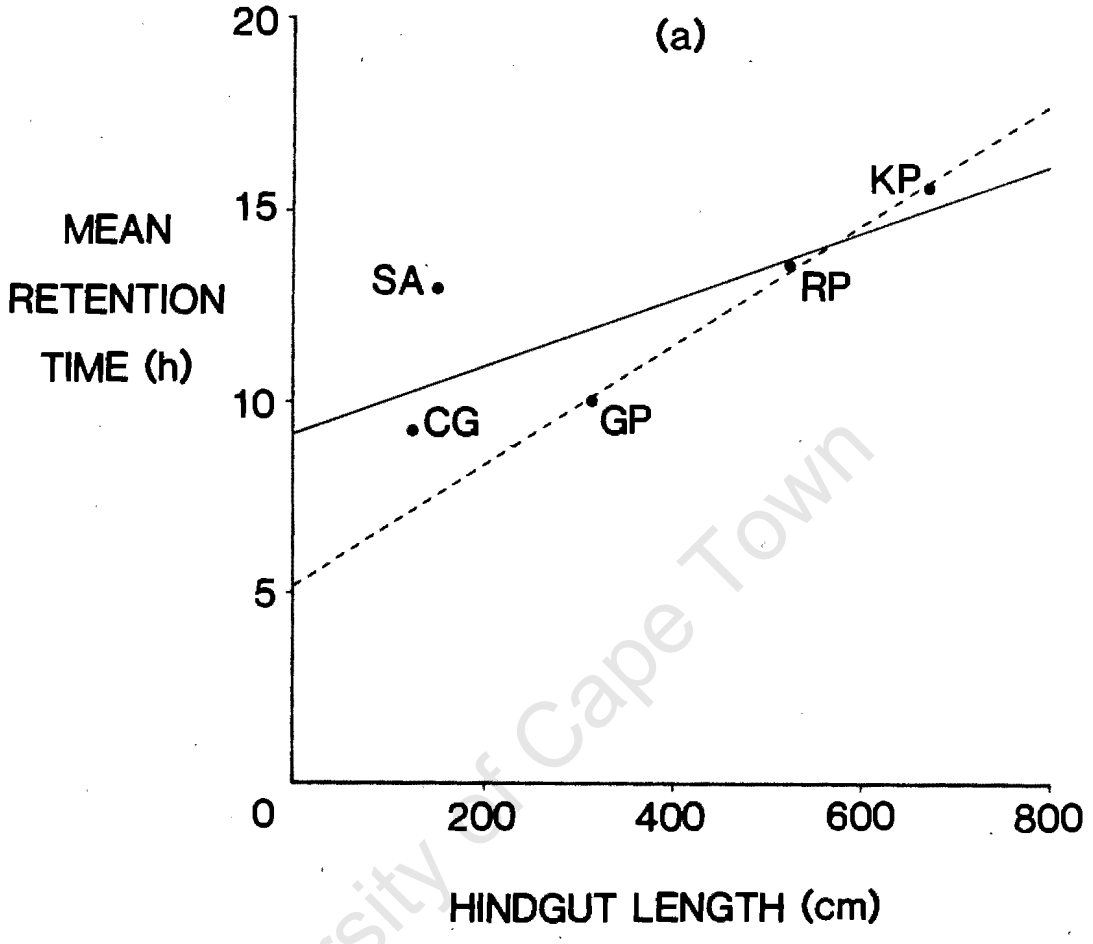

Figure 3.5. Regression of mean retention time of pilchard (hours) against hindgut length (cm) for the five seabird species studied. Abbreviations for the five species as for Fig. 3.1.

Equations:

– – – penguins only, $y = 0.016x + 5.116$, $r_1 = 0.999$, $P < 0.025$.

—— all species, $y = 0.009x + 9.171$, $r_3 = 0.776$, $P > 0.10$.

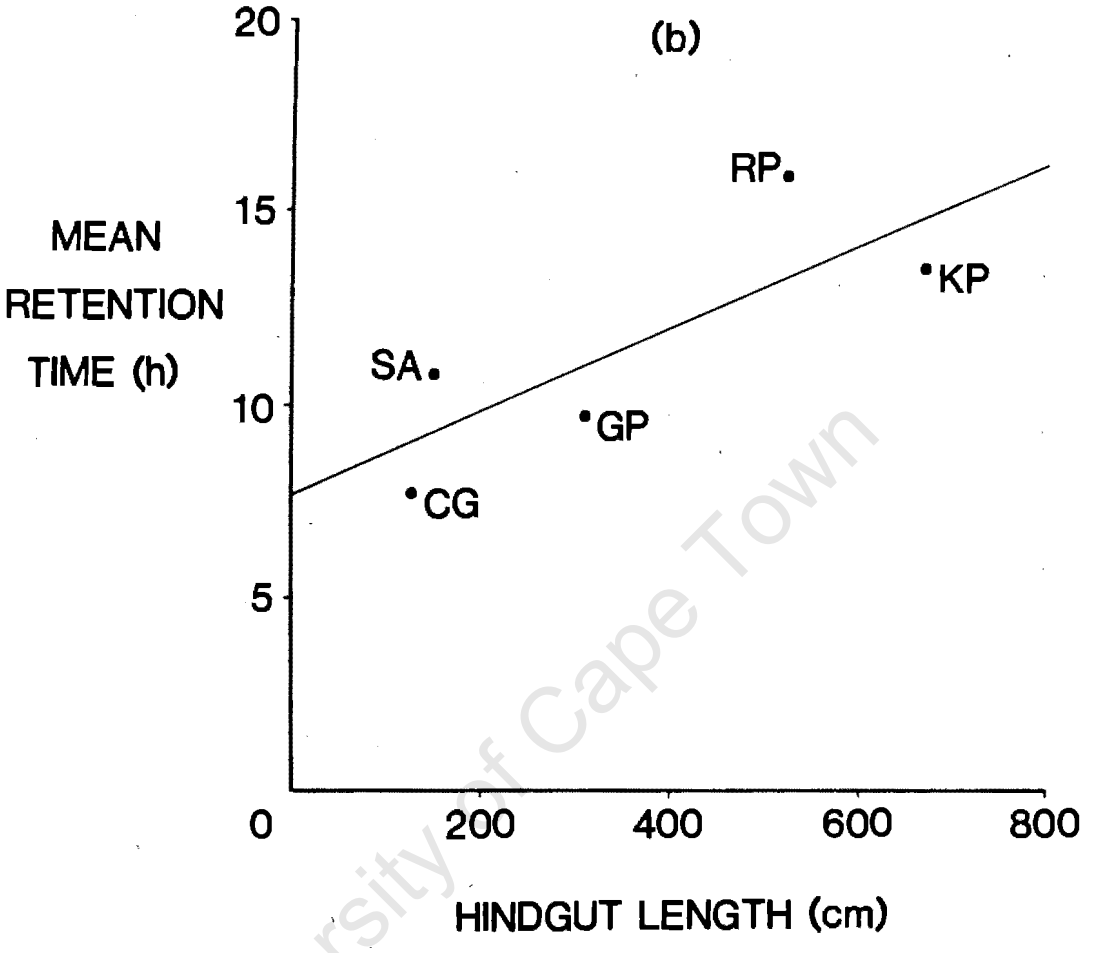

Figure 3.5 (ctd). Regression of mean retention time of prawns against hindgut length (cm) for the five seabird species studied. Species abbreviations as for Fig. 3.1.

Equation: $y = 0.011x + 12.612$, $r3 = 0.808$, $P < 0.05$.

mass, but for pilchard was positively correlated with hindgut length (Fig. 3.5). When the three penguin species were considered in isolation, correlations between mean retention times of pilchard and hindgut length were highly significant ($r_1 = 0.999$, $P < 0.025$). However, inclusion of the Cape Gannet and Sooty Albatross in this regression resulted in a non-significant correlation (Fig. 3.5). The interrelationship between body mass, hindgut length and hindgut volume (independent variables) necessitated the use of partial correlation (Zar, 1974) to isolate the primary determinant of mean retention times of each food type (the dependent variable). All seabird species were considered together. For birds fed pilchard, hindgut length accounted for a significant (56.51%) proportion of the total variance in mean retention time (58.51%) attributable to these three parameters ($r_{26} = 0.75$, $P < 0.001$). Mean retention time for squid was also primarily determined by hindgut length (53.01% of total variance, $r_{25} = 0.73$, $P < 0.001$), and although body mass accounted for 12.59% of the total variance in this case, the correlation was not significant. Amongst prawn-fed birds, hindgut length was again the only significant determinant of mean retention time (50.25% of the total variance, $r_{25} = 0.71$, $P < 0.001$).

Mean retention time and moult

Pre-breeding Rockhopper Penguins excreted pilchard significantly faster than did their conspecifics that had just completed moult (after 6 hours, $U_{6,6} = 34$, $P \leq 0.01$; after 24 hours, $U_{6,6} = 31$, $P \leq 0.05$). However, overall mean retention times of this food calculated after 48 hours ($t = 16.31 \pm 1.78$ hours, and $t = 15.08 \pm 2.38$ hours for pre-breeders and post-moulters, respectively) were not affected by moult. There were no differences between squid-fed birds from the two groups. For prawn-fed birds, mean retention times calculated after 12 hours, and after all time intervals from 18 hours onwards, were significantly longer in post-moult than in pre-breeding birds (after 48 hours, $t = 26.47 \pm 0.24$ hours and 20.20 ± 1.27 hours for the two groups respectively; $U_{4,6} = 24$, $P \leq 0.01$ for all time intervals). For all interspecific comparisons, data for the pre-breeding Rockhoppers

were used, because none of the other seabird species studied had recently completed moult.

The influence of meal size on mean retention times

Statistical comparisons between mean retention times for King Penguins fed different-sized meals of pilchard indicated that after 18 hours retention times did not differ between the single meals weighing 350 g, 750 g and 1000 g. Initially, the smallest meal (350 g) was excreted significantly faster than the largest (1000 g). Mean retention times for the 24- and 72-hour time intervals were significantly longer for the birds fed two 300 g meals than for those fed single meal of 1000 g ($H = 8.6023$, $P < 0.05$, $Y_m/S_m = 2.8257$, $P < 0.05$; and $U_{3,6} = 18$, $P < 0.025$ for the two time intervals, respectively).

Comparison of the carmine and gravimetric methods of measuring passage rate

Mean retention times (in hours, calculated 48 hours after feeding) of pilchard, squid and prawn meals fed to Sooty Albatrosses were 13.99 ± 2.38, 15.27 ± 3.39 and 14.03 ± 2.25 respectively when the carmine method was used, and 16.69 ± 2.25, 13.46 ± 0.55 and 17.33 ± 3.76 when the gravimetric method was used. None of the differences between estimates obtained using the two measurements was significant.

Cumulative excretion of solid and aqueous digesta

Initial excretion of pilchard was most rapid in the Cape Gannet (Fig. 3.6). Differing times to completion of feeding trials preclude direct statistical comparisons of cumulative faeces weights at corresponding time intervals, between species and between food types.

A greater proportion of the aqueous marker than of the solid digesta was excreted by both Sooty Albatrosses and Rockhopper Penguins in the first 20 hours after feeding (Fig. 3.8). Mean retention times of the aqueous marker fed to these two species were 6.3 and 3.8 hours, respectively (see Chapter 5). Corresponding mean retention times of solid digesta in Sooty Albatrosses and Rockhopper Penguins respectively were 16.7 and 16.3 hours for pilchard, 13.5 and 14.0 hours for

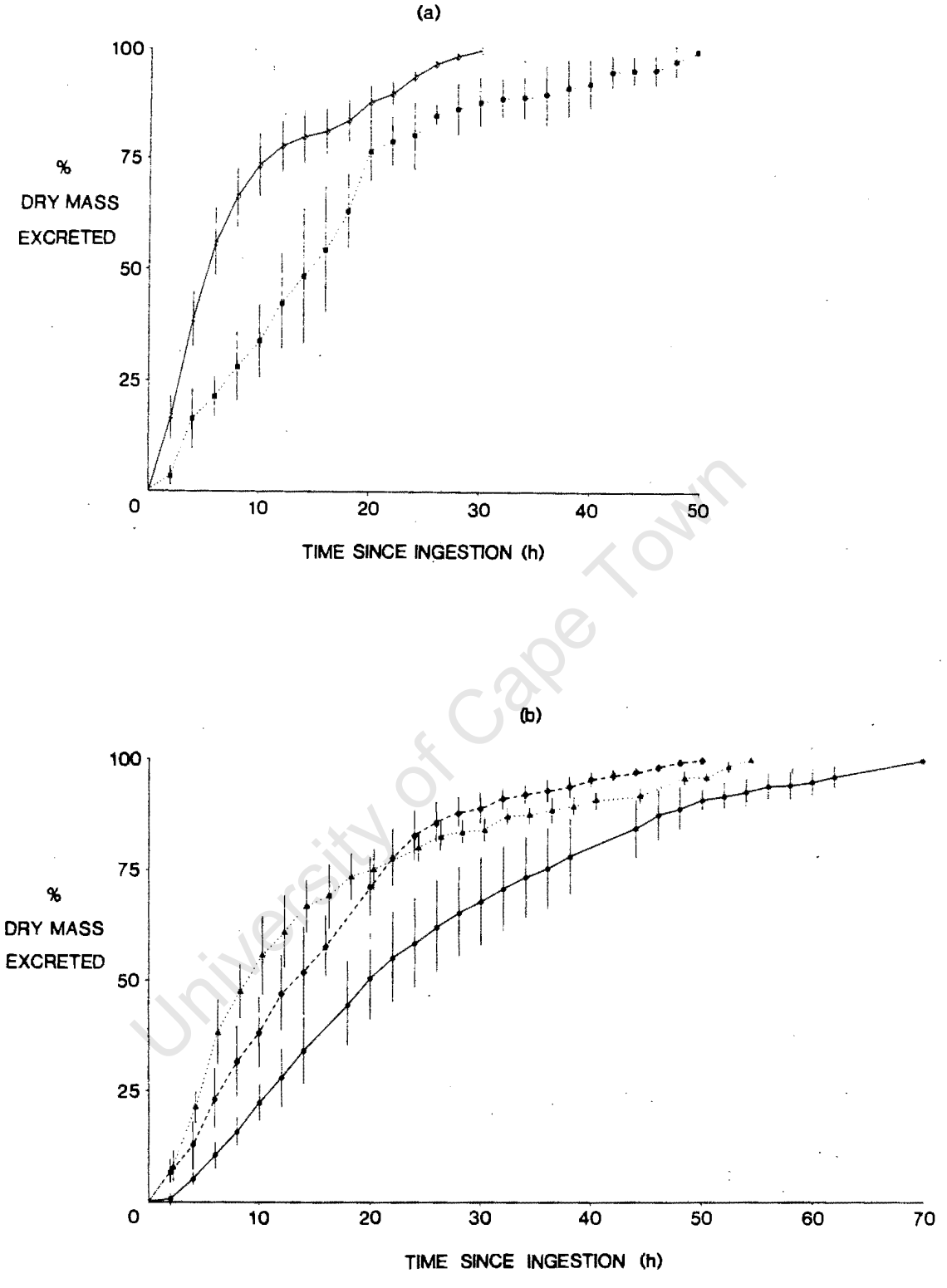

Figure 3.6. Cumulative percentages of faeces (dry wt, g) excreted by Cape Gannets (◇—◇) and Sooty Albatrosses (■····■) (a), and by Rockhopper (◆--), Gentoo (▲····▲) and King (●—●) penguins (b) after a pilchard meal.

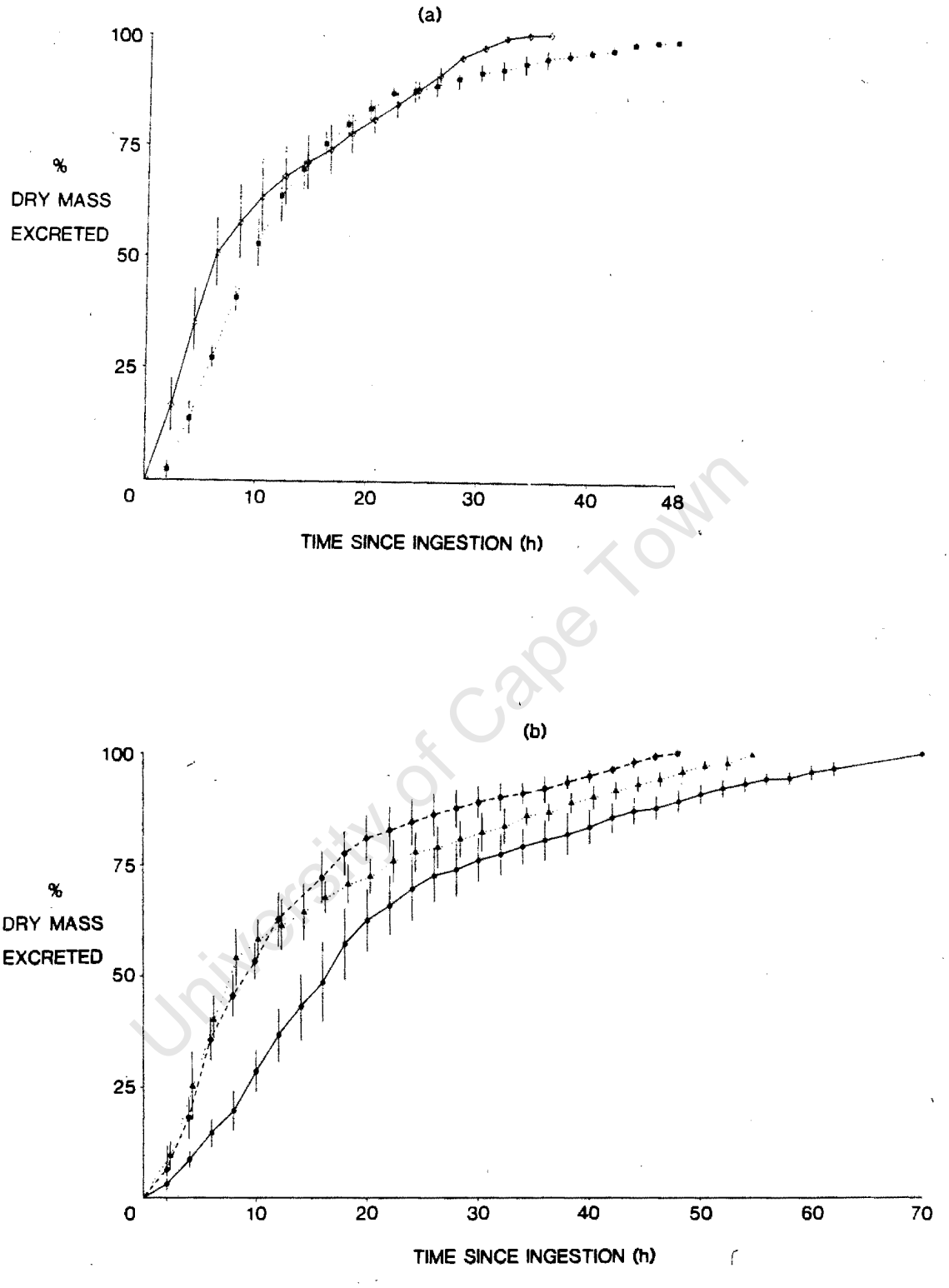

Figure 3.7. Cumulative percentages of faeces (dry wt, g) excreted by Cape Gannets and Sooty Albatrosses (a), and by Rockhopper, Gentoo and King penguins (b) after a squid meal. Symbols as for Fig. 3.6.

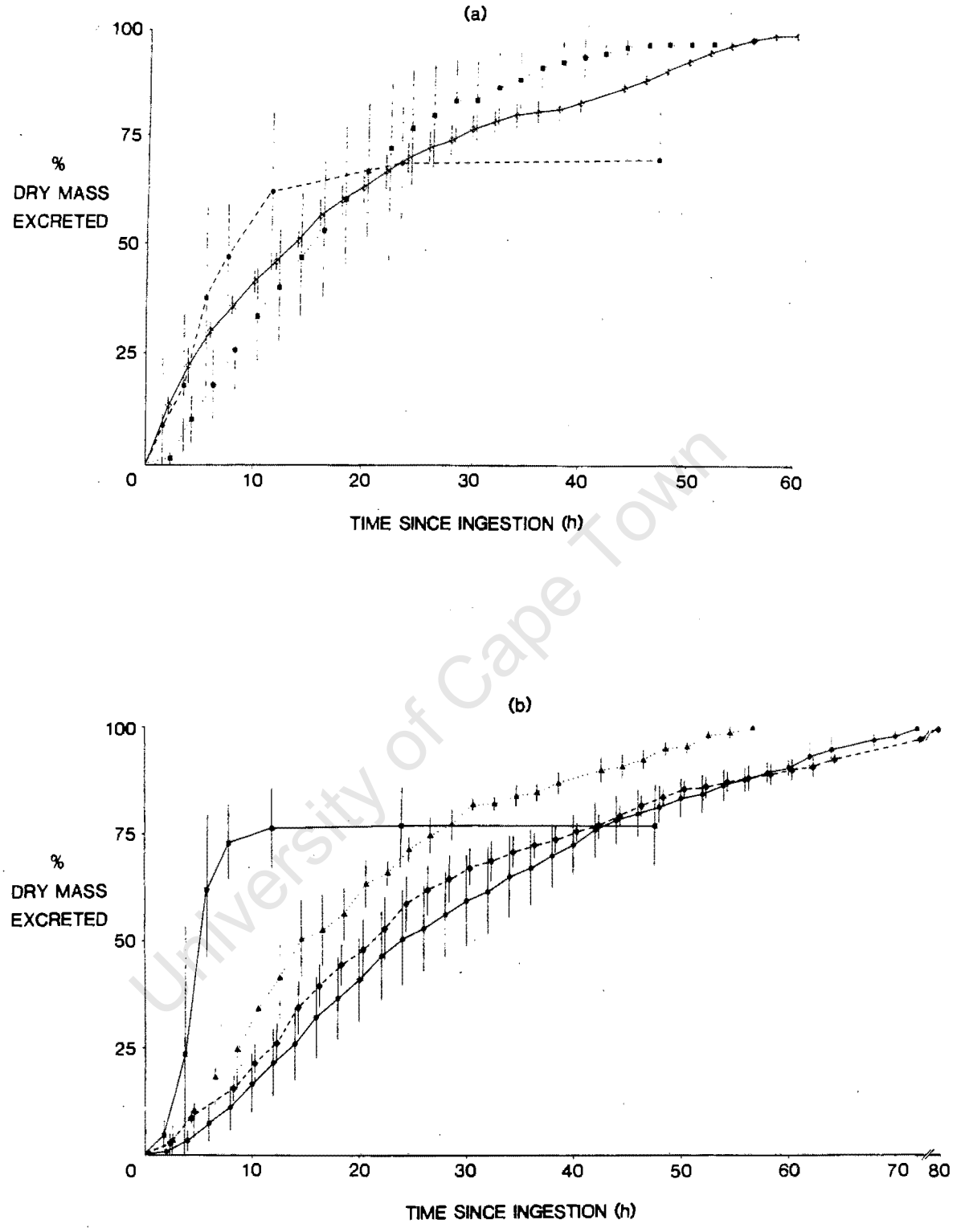

Figure 3.8. Cumulative percentages of faeces (dry wt, g) excreted by Cape Gannets and Sooty Albatrosses (a), and by Rockhopper, Gentoo and King penguins (b) after a prawn meal. Symbols as for Fig. 3.6 with the addition of

●- - ● : cumulative % aqueous marker ([^{3}H] PEG) excreted by Sooty Albatrosses and

■——■ : by Rockhopper Penguins.

squid, and 17.3 and 20.2 hours for prawns (Tables 3.2, 3.3 and 3.4). Initially rapid aqueous excretion in both species results in exponential excretion curves, whereas the corresponding excretion curves for solid digesta are more linear (Figs 3.6, 3.7 and 3.8).

Gastric evacuation times

Cape Gannets exhibited the shortest gastric evacuation times of all three foods for all seabirds studied (Table 3.6). Sooty Albatrosses showed no differences between food types, but for all other species, prey ranking in order of decreasing digestibility was pilchard, followed by squid and then prawn.

Quantitative gastric evacuation data obtained by sequential stomach pumping of Sooty Albatrosses could not be used to calculate actual mean retention times of gastric digesta for comparison with mean retention times for the entire gut, because the birds were not stomach pumped at two-hourly intervals. Mean gastric retention times calculated for the three foods *in vitro* (see Chapter 1) range from 21 to 46% of mean total gut retention times for pelagic fish (pilchard or anchovy). Corresponding values for squid and crustacean muscle expressed as percentages of total gut retention times of squid and prawn are 30 - 57% and 33 - 47% respectively. Mean retention times for prawn tails digested *in vitro* are similar in value to observed total gut retention times for this food.

DISCUSSION

Gastrointestinal transit in relation to foraging method and gut length

The weight-saving advantages of rapid gastro-intestinal food passage in flying birds have been discussed by Sibly (1981), who states that "a bird's digestive strategy should minimize the weight carried". Body mass exerts an indirect influence over mean retention times of all three food types, because both hindgut length (the primary determinant of mean retention time), and hindgut volume, scale with body mass. In terrestrial herbivores, the relationship between gut capacity (ie: wet mass

Table 3.6. Times (h) to complete gastric evacuation of three food types fed to Cape Gannets, Sooty Albatrosses, and Rockhopper and King Penguins, determined by inspection using a fiber-optic gastroscope. Figures in parentheses are times to evacuation of prey "hard parts": pilchard bones, squid pens and prawn exoskeletons.

Species	Prey type		
	Pilchard	Squid	Prawn
Cape Gannet	12 (16)	14 (16)	30 (36)
Sooty Albatross	24 (24)	24 (30)	30 (36)
Rockhopper Penguin	24 (36)	30 (30)	24 (48)
King Penguin	18 (24)	24 (24)	n.d.

of gut contents) and body mass sets evolutionary limits on body size (Demment, 1983; Demment and van Soest, 1985), because metabolic rate scales with (body mass)$^{0.75}$, and gut capacity with (body mass)$^{1.03}$. Larger animals are thus better able to meet their metabolic needs, because the ratio of gut capacity to energy requirements increases with body size. The same may be true of penguins, for which a similar ratio applies, but gut capacity increases with a lower exponent of body mass in flying seabirds. Field metabolic rate in seabirds generally scales with (body mass)$^{0.70}$ (Nagy, 1987). Results of the present study show that hindgut volume scales with (body mass)$^{1.09}$ for all seabird species, with (body mass)$^{0.81}$ for flying species, and with (body mass)$^{1.27}$ for penguins. The different exponents indicate that as body size increases, mass-specific gut volume decreases in flying species, whereas the reverse is true for penguins. Relationships between gut length and body mass show the same pattern. Because none of the above regressions differ significantly, the trends that they demonstrate can only be be confirmed or disproven by the incorporation of gut measurements for small penguins and larger flying seabirds into the data set.

The scaling of gut size to body mass in flying species may reflect allocation of an increasing proportion of body mass to skeletal and muscular flight equipment as bird body size increases, in response to aerodynamic demands. This may result in lower scaling exponents for flying seabirds than for terrestrial animals such as the herbivores studied by Demment and Van Soest (1985). The upper limit to flying bird body mass (approximately 12 kg) is set by the power that can be delivered by flight muscles (Pennycuick, 1975). As birds such as the larger albatrosses approach this limit, the interaction of mass-specific energy requirements, digestive capacity and muscle power output must influence their flying, hence foraging abilities. Seabirds exhibit the widest range of body sizes and foraging methods of all groups of birds, and the flightless penguins offer another dimension for comparison. Scaling of seabird digestive capacity to body size has received little attention, and has both ecological and evolutionary significance.

Do penguins exhibit slower gut passage rates than flying species? Because mean retention time is only indirectly correlated with body mass through hindgut length, weight-related trends in passage times of digesta are not immediately apparent from the present data set. For instance, one would expect both Cape Gannets and Sooty Albatrosses to exhibit shorter mean retention times than do the three penguin species. Although Cape Gannets show significantly shorter mean retention times than do King Penguins, Gentoo Penguins consistently retain food for shorter periods than do Sooty Albatrosses, although seldom significantly so. Allometry may prove more meaningful than direct comparisons between penguins and other seabirds.

The interspecific differences in mean retention times within the penguins are related to differences in gut lengths within this group. Significant correlations between hindgut lengths of the three penguins and mean retention times of pilchard, strongly suggest this. The lower correlation coefficient for this regression upon inclusion of data for the two flying species indicates that separate regressions for flying and non-flying seabirds might yield better correlations than the use of pooled data, but again, more species must be included in this comparison in order to assess the extent of variation within the two groups.

Mean retention time of digesta is apparently related to foraging trip duration. For the penguins, the ranking of mean retention times in order of increasing length (Gentoo, Rockhopper, King) is the same as that in order of increasing foraging range. Mean foraging ranges in kilometers for the three penguins in the same order (ranges in parentheses) are 14 (1 - 103), 33 (2 - 137) and 301 (75 - 902) (Adams and Brown, 1989). More importantly, mean foraging trip durations for Gentoo, Rockhopper and King Penguins feeding large or medium-sized chicks are 0.6, 3.0 and 4.0 days respectively (Adams and Brown, 1989). For the Cape Gannet and Sooty Albatross, foraging trips last for an average of 0.79 and 1.88 days, respectively (Berruti, 1977; Navarro and Adams, ms). For all species together, mean retention times of pilchard calculated 30 hours after feeding (y) are positively correlated with

foraging trip duration in days (x) ($y = 8.661 + 1.767x$; $r_3 = 0.966$; $P < 0.01$). A corresponding regression for mean retention time of squid was not significant, but mean retention times of prawn (calculated after 48 h) were also significantly correlated with foraging trip duration ($y = 15.594 + 1.608x$, $r_3 = 0.903$, $P < 0.01$).

Mean retention times and field metabolic rates

Data for field metabolic rates (FMR's) are unavailable for Rockhopper Penguins and Sooty Albatrosses, and published FMR estimates for Gentoo Penguins do not distinguish between foraging birds and birds at the nest (Davis *et al.*, 1989). However, comparison of at-sea FMR's estimated using the doubly-labelled water technique for Grey-headed Albatrosses (*Diomedea chrysostoma*), which are sympatric with Sooty Albatrosses at Marion Island, with corresponding estimates for the Cape Gannet, indicates that Cape Gannets expend more energy while foraging than do albatrosses. At-sea FMR's for the two species respectively are: 1901.1 and 654.8 kJ/kg body mass/day (Adams and Brown, 1984; Adams *et al.*, ms; Costa and Prince, 1987). The difference may be a result of flying technique: the gliding flight of albatrosses (Pennycuick, 1982) is probably less energy-expensive than the alternation of flapping and gliding employed by the gannet. Although the comparison remains speculative in the absence of FMR data for the Sooty Albatross, it may be that faster gut passage rates in the gannet are an adaptation to reduce meal mass rapidly, important for a species using an energetically expensive mode of flight.

Mean retention time in relation to natural diet

Of all the species studied, the African resident Cape Gannet was the only one which retained squid in the gut for longer than it did pilchard. The four seabirds which breed at Southern Ocean islands (the Sooty Albatross and the three penguins) retained squid for shorter periods than they did pilchard, despite the relative indigestibility of the former food type *in vitro* (Chapter 1). The Cape Gannet is a specialist pelagic fish feeder and seldom eats squid, whereas squid are

abundant in the Southern Ocean and are eaten frequently by *Phoebetria* albatrossess (Thomas, 1982; Berruti, 1977; Berruti and Harcus, 1978) and the three penguin species (Adams and Brown, 1989). The Southern Ocean species may be adapted to pass squid rapidly through the intestine. Gastric digestion of this food in sub-Antarctic seabirds may be facilitated by the enzyme collagenase, a protease which would help digest the collagen-rich (Bone *et al.*, 1981) muscle tissue of squid.

Retention times of solid and aqueous digesta

Jobling (1986) predicted that gastric emptying rates of small, easily digested food particles in fish would conform most closely to an exponential function, whereas larger food particles of higher energy value would be evacuated in a linear fashion with time. The same may be true of seabirds: the few studies comparing gut passage rates of different dietary components in seabirds indicate that aqueous digesta are evacuated more rapidly than are lipids from both the stomach (Duke *et al*, 1989; Roby *et al.*, 1989) and the entire gut (Roby *et al.*, 1989; Chapter 5). Data presented in this chapter confirm differential transit times of solid and aqueous digesta through seabird guts. The energetic implications of differential passage rates of lipid and aqueous digesta in procellariiforms have been discussed by Cheah and Hansen (1970), Warham (1977) and Roby *et al.* (1989) (see also Chapter 5), but the implications for microbial digestion in seabirds have not.

Rapid water passage through seabird guts may influence their suitability as homes for bacteria. Studies of the gut flora of seabirds have been largely descriptive (Sieburth, 1959; McBee, 1960; Soucek and Mushin, 1970), although the role of microbes in the digestion of chitin has recently attracted attention (Chapter 6). There is evidence for microbial fermentation of chitin in baleen whale forestomachs (Herwig *et al*, 1984).

Gasaway *et al.* (1975) studied relative passage rates of dry matter and liquids through Rock Ptarmigan (*Lagopus mutus*) guts, and found that water was retained in the cecum, presumably to facilitate the microbial cellulose fermentation which occurs there, and which yields volatile fatty acids (Gasaway, 1976a, b, c). In the

absence of enlarged ceca, microbial cellulose fermentation may take place in the hindgut, as it does in the Emu (*Dromaius novaehollandiae*) (Herd and Dawson, 1984). Like seabirds, this species evacuates water more rapidly from the gut than solid digesta, with mean retention times of 4.1 ± 0.2 and 5.5 ± 0.4 hours for the two phases respectively (Herd and Dawson, 1984). However, the difference between mean retention times of the two dietary components is less marked in this species than in seabirds. The implications of relatively rapid evacuation of water from seabird guts for microbial digestion need to be assessed.

Prey digestibility in relation to mean retention times

The ranking of the three food types used in this study according to decreasing *in vitro* digestibility (fish, squid, crustaceans, see Chapter 1) is reflected by times to complete gastric evacuation of these three foods in the five seabird species studied here, although gastric evacuation times obtained using the gastroscope inspection method are relative rather than quantitative. Sequential stomach pumping of Sooty Albatrosses (Chapter 2), and White-chinned Petrels and Jackass Penguins (Wilson *et al.*, 1985; Jackson and Ryan, 1986) suggests the same prey digestibility ranking as that obtained using the gastroscope inspection method.

The individual prawns used in the feeding experiments were larger and had thicker exoskeletons than the crustaceans naturally eaten by the species studied. Much of the prawn exoskeleton may have been retained for longer than the duration of the feeding experiments, or was regurgitated as undigested pellets. Consequently, mean retention times and excretion curves probably represent prawn flesh rather than exoskeleton. This is further suggested by the fact that *in vitro* mean retention times of prawn tails including exoskeleton equalled or exceeded total *in vivo* gut retention times of this food (Chapter 1). Conclusions about gut passage rates of crustaceans relative to other foods drawn on the strength of data presented here should therefore be treated with caution.

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Appendix 3.1. Values of Kruskal-Wallace test statistics for interspecific pairwise comparisons of mean retention times in birds fed fish. < indicates which of the two species exhibited a lower mean retention time. Species names abbreviated for clarity DF: degrees of freedom.

Time interval	Species pair	DF	H	P <	Y_m/S_m	P <
6 h	Gannet < King	5	22.325	0.001	3.465	0.05
12 h	Gannet < King	5	23.698	0.001	4.337	0.01
18 h	Gannet < King	4	23.188	0.001	4.4709	0.01
	Gannet < Sooty	"	"	"	3.243	0.05
	Gentoo < King	"	"	"	3.050	0.05
24 h	Gannet < King	5	26.620	0.001	4.300	0.01
	Gentoo < King	"	"	"	3.754	0.01
	Gannet < Rockhopper	"	"	"	3.203	0.05
30 h	Gannet < King	5	27.755	0.001	4.389	0.01
	Gentoo < King	"	"	"	4.220	0.01
36 h	Gentoo < King	4	19.760	0.001	4.413	0.01
42 h	Gentoo < Sooty	3	12.414	0.01	3.227	0.05
	Gentoo < Rockhopper	"	"	"	2.8633	0.05
48 h	Gentoo < King	4	17.298	0.005	3.905	0.01
52 h	Gentoo < King	2	12.593	0.005	3.540	0.01
54 h	Gentoo < King	$U_{6,6} = 36, P < 0.005$				

Appendix 3.2. Values of Kruskal-Wallace test statistics for interspecific pairwise comparisons of mean retention times in birds fed squid. < indicates which of the two species exhibited a lower mean retention time. DF: degrees of freedom.

Time interval	Species pair	DF	H	P <	Y_m/S_m	P <
6 h	Gannet < Sooty	5	15.314	0.01	3.284	0.05
12 h	Gannet < Sooty	5	25.863	0.001	3.632	0.01
	Gentoo < Sooty	"	"	"	3.383	0.05
	Gannet < King	"	"	"	3.735	0.01
	Gentoo < King	"	"	"	3.478	0.01
18 h	Gannet < King	5	25.433	0.001	4.228	0.01
	Gentoo < King	"	"	"	4.002	0.01
24 h	Gannet < King	5	23.176	0.001	3.796	0.01
	Gentoo < King	"	"	"	4.040	0.01
30 h	Gentoo < King	5	21.265	0.001	4.151	0.01
36 h	Gentoo < King	3	15.195	0.005	3.815	0.01
42 h	Gentoo < King	3	11.826	0.01	3.262	0.01
48 h	Sooty < King	3	10.568	0.025	2.940	0.05
54 h	Gentoo < King	$U_{5,6} = 29, P < 0.005$				

Appendix 3.3. Values of Kruskal-Wallace test statistics for interspecific pairwise comparisons of mean retention times in birds fed prawns. < indicates which of the two species exhibited a lower mean retention time. DF: degrees of freedom.

Time interval	Species pair	DF	H	P <	Y_m/S_m	P <
12 h	Gannet < King	5	19.350	0.005	3.298	0.05
18 h	Gannet < King	5	17.630	0.005	3.698	0.01
	Gannet < Rockhopper	"	"	"	3.155	0.05
24 h	Gannet < King	5	22.861	0.001	3.411	0.05
30 h	Gannet < King	4	20.784	0.001	3.088	0.05
36 h	Gannet < King	5	22.518	0.001	3.333	0.05
54 h	Gannet < King	3	12.699	0.01	3.179	0.05
60 h	Sooty < King	2	9.437	0.01	3.031	0.05

CHAPTER 4

SEABIRD ASSIMILATION EFFICIENCY IN RELATION TO PREY COMPOSITION

(In part) *Comp. Biochem. Physiol.* 85A:301-303. (1986)

SUMMARY

Energy, lipid, nitrogen and calcium assimilation efficiencies (AE), were experimentally determined for seven seabird species: a sulid, three procellariiforms and three spheniscids. Metabolizable energy coefficients (MEC) of fish, squid and crustaceans were also calculated, and compared with values predicted on the basis of food composition and endogenous energy and nitrogen losses.

MEC does not appear to be higher for prey types favoured by naturally-foraging birds, and is remarkably similar across all species and all foods despite differences in food composition and in the dietary preferences of the birds. The overall mean value of MEC (75%) may represent the optimal value for marine predatory birds.

Meal mass may be inversely related to energy AE, and the relationship between meal mass and MEC shows that the wet mass of meals fed to experimental birds should not be below 6.5% of bird body mass. Differences in bird body mass and in the duration of experiments may influence commonly used estimates of AE. Interspecific comparisons of MEC, a parameter which takes endogenous losses of energy and nitrogen into account, are thus more meaningful than comparisons of AE calculated simply on the basis of energy intake and excretion. The assimilation efficiency of 75% hitherto frequently assumed in seabird consumption and energetics models, is perfectly adequate.

Surprisingly, energy AE and MEC are unrelated to mean retention times of solid digesta in seabird guts. MEC of squid is correlated with hindgut surface area, which in turn scales with body mass. Measurements of villus area for seabird guts are needed to investigate the relationship between AE, gut surface area, and body mass.

Immature albatrosses assimilate nitrogen and calcium more efficiently than do adult conspecifics, presumably to supply growth needs. Penguins that have just finished moult exhibit higher MEC's, and AE's of calcium and nitrogen, than do pre-laying birds under less energetic stress, presumably to make good the energy deficit incurred during moult.

INTRODUCTION

Experimentally-determined values of energy utilization efficiency, or assimilation efficiency, yield insight into digestive adaptations influencing the trophic niche of an animal (Karasov, in press). Seabird prey distribution is spatially and temporally unpredictable (Ricklefs, 1983), necessitating opportunistic foraging. In such a situation, maximally efficient digestion of a wide range of foods would be adaptive. However, interspecific dietary segregation has been documented for seabird communities, for example, at South Georgia (Croxall and Prince, 1980), the Prince Edward Islands (Adams and Brown, 1989), and the Washington State coast (Cody, 1973). Do seabirds specialize in assimilating their favoured prey more efficiently than other food types, or are all seabirds digestive opportunists? The present study addresses this question by inter- and intraspecific comparisons of assimilation efficiencies in seven seabird species.

Most studies on assimilation efficiency in seabirds have been restricted to single food types (Dunn, 1975; Cooper, 1977, 1978, 1980; Adams, 1984; Davis *et al.*, 1989). Within species, there is evidence for variation in energy assimilation efficiency between food types (Batchelor and Ross, 1984; Heath and Randall, 1985; Nelsen and Brandl, 1988). The variation may be partly a consequence of prey composition (Karasov, in press). In this study, I assess the extent to which observed metabolizable energy coefficients (MEC) of different food types are predictable on the basis of food composition (Karasov, in press). If observed MEC values are in close agreement with estimates predicted assuming that prey tissues such as crustacean exoskeleton are indigestible, seabirds are probably digestive opportunists, because digestive specialization should be reflected in the ability to assimilate prey components such as chitin.

The relationship between assimilation efficiency and gastrointestinal passage rates, and food preference, has been studied in frugivorous passerines (Sorensen, 1984; Martínez del Rio *et al.*, 1989) and shorebirds (Castro *et al.*, 1989), but has not been documented in seabirds. Moreover, models of digestion assume that the net

amount of energy gained from food is proportional to the amount of time that food resides in the gut (Sibly, 1981), but lipid assimilation efficiency in seabirds is not simply a linear function of time (see Chapter 5). Assessment of the value of different foods to seabirds should take into account a combination of digestibility, total gut passage rate, and assimilation efficiency.

Published studies present contradictory evidence relating maturity to assimilation efficiency. Studies on passerines (Westerterp, 1973; Blem, 1975) ducks (Cain, 1976), and on nestling Double-crested Cormorants *Phalacrocorax auritus* (Dunn, 1975), Jackass Penguins *Spheniscus demersus* (Heath and Randall, 1985) and Black-headed Gulls *Larus ridibundus* (Nelsen and Brandl, 1988) indicate that energy assimilation efficiency increases with age. In contrast, Cooper (1977, 1978, 1980) found no such trend in Jackass Penguins, Cape Gannets *Morus capensis* and Great White Pelicans *Pelecanus onocrotalus*. Differences in calcium assimilation efficiency between adult and nestling White-breasted Cormorants *P. carbo sinensis* have been suggested but not proven by van Dobben (1952). Here, I compare assimilation efficiencies of various dietary components in adult and fledgling Sooty Albatrosses (*Phoebetria fusca*), to investigate the possibility that immature seabirds have lower assimilation efficiencies.

In adult passerines, energy assimilation efficiency varies in response to the seasonally fluctuating energetic demands of migration (King, 1961; Bairlein, 1985), and might also be expected to do so during the moult cycles of penguins. Penguin moult is a period of severe energetic stress (Brown, 1985), and increased energy assimilation efficiency immediately following moult would be adaptive, permitting restocking of energy reserves. By comparing pre-breeding Rockhopper Penguins (*Eudyptes chrysocome*) with individuals that had just finished moulting, I tested the validity of this speculation.

Finally, measurements of energy assimilation efficiencies permit conversion of energy requirements of free-living seabirds to estimates of their daily food requirements (Miller and Reinecke, 1984), which are of fundamental importance to

models of energy and nutrient flow. Translation of energy into food requirements (eg: Furness, 1978; Furness and Cooper, 1982; Brown, 1989) has often relied on assumed values of assimilation efficiency. In reviewing the status of energetics models of seabird populations, Wiens (1984) highlighted the inadequacy of information on assimilation efficiencies. This study presents energy assimilation efficiencies for a suite of seabirds including procellariiforms and penguins which, by virtue of their large population and body sizes, are important marine predators.

MATERIALS AND METHODS

Feeding experiments

Seven seabird species with a range of natural diets were selected for the feeding experiments: the Cape Gannet *Morus capensis* (Sulidae), Blue Petrel *Halobaena caerulea*, White-chinned Petrel *Procellaria aequinoctialis* and Sooty Albatross *Phoebetria fusca* (Procellariidae), and the Rockhopper *Eudyptes chrysocome*, Gentoo *Pygoscelis papua* and King *Aptenodytes patagonicus* penguins (Spheniscidae). The experimental procedure is described in Chapter 3. The birds were fed one of three food types: fish, squid or crustaceans. Within the fish category, White-chinned Petrels were fed light-fish *Maurolicus muelleri*, and the Sooty Albatrosses and Blue Petrels were fed anchovy *Engraulis japonicus capensis*. All other seabirds were fed pilchard *Sardinops ocellatus*. The squid *Loligo vulgaris reynaudii* was used throughout, and crustacean food was either Antarctic Krill *Euphausia superba*, or prawn *Penaeus indicus*. Prawns were used as a substitute for krill, which was not available throughout the study. All food was stored frozen, and thawed immediately before use.

The effect of multiple meals and different meal sizes on assimilation efficiency in King Penguins was assessed in a series of feeding trials with differing meal sizes and frequencies (see also Chapter 3).

Time at the study site (Marion Island, 46°52'S, 37°51'E) was limited to three to six week takeover periods (see Chapter 3), and individual birds could not be kept in

captivity long enough for habituation to occur. *Ad libitum* feeding was therefore not attempted, and birds were kept for as short a time as possible in order to minimize the influence of capture stress on their digestive processes. Avian assimilation efficiency may initially drop in response to altered diet, before returning to original levels (Levey and Karasov, 1989). By keeping birds captive for as short a time as possible, and by feeding them only one meal, I hoped to ensure that the change to an artificial diet influenced assimilation efficiencies of all food types equally.

Each bird was released as soon as the meal had passed throught the gastrointestinal tract (see Chapter 3). Although meal sizes were within the range of published meal masses eaten by free-living birds (Thomas, 1982; Batchelor and Ross, 1984; Berruti *et al.*, 1985; Steele and Klages, 1986; Adams and Klages, 1987; Adams and Wilson, 1987; Brown and Klages, 1987), the birds regurgitated if fed enough food in a single meal to meet their energy requirements for the two to four days of the feeding experiments. Birds were weighed before and after the feeding trials, and most individuals lost mass during the experiments. In the light of the results of the experiment investigating assimilation efficiencies in King Penguins fed different-sized meals, I assumed that the short-term body mass loss suffered by most experimental birds did not influence the results of the balance studies (see discussion below). Estimates of nitrogen-corrected apparent metabolizable energy provide a basis for comparisons independent of endogenous nitrogen loss between birds that are not in nutritional equilibrium.

For the three penguin species and the Cape Gannet, pooled faecal samples from gastrointestinal passage rate experiments (Chapter 3) were used. Separate feeding trials involving a single faecal collection were carried out for the three procellariiforms. In these experiments, pre-weighed aluminium foil trays were used for the collection and drying of faeces.

Laboratory analyses

Food and faecal samples were dried to constant mass for three - five days in a chamber equipped with a dehumidifier. The temparature was kept at 45°C to

prevent volatilization of lipids. The samples were then homogenized using a pestle and mortar and an electric coffee mill, and stored dry in sealed jars. Analyses were carried out on duplicate subsamples, and the results expressed as percentages of dry mass. The energetic value of representative samples of all food types and of faeces was determined using a Phillipson CP 500 microbomb calorimeter. The nitrogen content of food and faeces was determined by the Kjeldahl method (Dowgiallo, 1975).

The evidence for nitrogen loss from bird faeces during oven-drying is contradictory: Blem (1968) and Dale *et al.* (1985) found no significant nitrogen losses from oven-dried House Sparrow *Passer domesticus* and rooster *Gallus domesticus* excreta respectively. However, Herd (1982) found that oven-dried Emu *Dromaius novaehollandiae* excreta contained 20% less nitrogen than did samples of the same excreta after freeze-drying. He concluded that nitrogen losses are proportionally greater in faeces with low (0.013% of dry mass) than with high (0.14%) nitrogen content. The seabird faeces in this study comprised between 5 and 20% nitrogen per unit dry mass, one to two orders of magnitude greater than corresponding values for Emu faeces (Herd, 1982). I therefore assumed that oven-drying had an insignificant effect on the nitrogen content of the faecal samples in this study.

Because water was added to faecal samples that were collected in the gastrointestinal passage rate experiments, and then pooled for this study, these samples took longer to dry than did samples obtained from a single faecal collection. To prevent bacterial breakdown of lipids and enhanced nitrogen loss during drying, measured quantities of sodium azide (NaN_3) were added to the pooled faecal samples. The mass of nitrogen thus added to the faeces was subtracted from the results of the Kjeldahl analyses before calculation of energy assimilation efficiency (AE_e), energy assimilation efficiency corrected for nitrogen retention (AE_{eN}) and nitrogen assmilation efficiency (AE_N).

The total lipid content of food and faecal samples was determined using ethanol

and a 50:50 mixture of petroleum ether and diethyl ether. Extractions were performed twice per duplicate subsample, and the extracts combined. The procedure was adapted from that detailed by the American Association of Analytical Chemists (Horwitz *et al.*, 1975).

The ash content of food and faecal samples was determined by combustion at 650°C for 16 hours. After weighing, the residue was dissolved in 5% HCl over a steam bath, diluted, and the calcium content determined using an atomic absorption spectrophotometer. Potassium chloride was used as the ionization suppressant for the nitrous oxide/acetylene flame.

The results of the above analyses are expressed as percentages of dry food mass.

Definition of terms

Throughout the text, the term "assimilation efficiency" refers to the Apparent Metabolizable Energy (AME) of the food (Miller and Reinecke, 1984), expressed as a percentage of the total energy ingested. Digestion studies of wild birds refer variously to "metabolic energy coefficient" (Davis *et al.*, 1989), "assimilation efficiency" (AE) (Nelsen and Brandl, 1988), "digestive efficiency" (Dunn, 1975; Cooper, 1977) and "utilization efficiency" (Adams, 1984; Karasov, in press; Levey and Karasov, 1989) or erroneously to "metabolic efficiency" (Heath and Randall, 1985), all determined by the same experimental procedure, and all synonymous with the term "energy assimilation efficiency" (AE_e) used throughout the present study. "Assimilation efficiency" is thus a physiological parameter applied to the experimental birds. It is important to note that estimates such as the above examples, and AE_e's presented below, refer to *apparent* values before correction for endogenous energy and nutrient losses. This point is often overlooked in the published literature on wild birds (Jackson, 1986; Karasov, in press).

When expressed as fractions per unit of food, rather than percentages of gross intake by experimental birds, estimates of apparent AE become the "apparent metabolizable energy coefficient" (MEC*) of the food, a term described by Kendeigh *et al.* (1977). Metabolizable energy (ME), a property of foodstuffs, is

commonly used in the extensive literature on domestic poultry (see Sibbald, 1982 for a review), and is defined by Miller and Reinecke (1984) as a "measure of the energy available to birds from their diet". The metabolizable energy coefficients (MEC's) referred to below are corrected for endogenous nitrogen and energy losses using the most appropriate data available.

Data analyses

Energy, lipid (AE_{lip}), calcium (AE_{Ca}) and nitrogen assimilation efficiencies were calculated using the formula:

$$AE = (T_{in} - T_{ex})/T_{in} \quad (1)$$

where T_{in} is the total quantity (kJ or g) ingested, and T_{ex} the total quantity excreted. The results of these calculations were expressed as percentages. Energy assimilation efficiencies corrected to zero nitrogen balance (AE_{eN}, also expressed as percentages) were calculated using the formula:

$$AE_{eN} = (E_{in} - E_{ex} - N)/E_{in} \quad (2)$$

where E_{in} is the total amount of energy ingested (kJ), E_{ex} the total amount of energy excreted (kJ), and N a nitrogen correction factor calculated using the formula:

$$N = (N_{in} - N_{ex}) \times 36.5 kJ.g^{-1} \quad (3)$$

N_{in} and N_{ex} respectively are the total amount of nitrogen ingested and excreted (g). The unit of N is thus kJ. The value $36.5 kJ.g^{-1}$ is the energy value of the mixture of nitrogenous components comprising chicken urine (Titus *et al.*, 1959). Sibbald (1982) considers this value to yield more accurate estimates of nitrogen energy balance than the figure 34.4 $kJ.g^{-1}$ assigned to urinary nitrogen by Hill and Anderson (1958), in the assumption that avian excretory products comprise only urea (see also Harris, 1966 and Miller and Reinecke, 1984). Values for AE_N are expressed as percentages. Karasov (in press) has developed a simple linear model

considered to have no refractory material, because procellariiform seabirds and penguins digest rather than regurgitate fish bones. Moreover, none of the birds used in this study regurgitated bones.

Values for the second term of Equation (4) were obtained from Kjeldahl analyses for each food type (see above). Finally, the energy value 21 kJ.(kg body mass)$^{-0.75}$.d^{-1} was used to estimate endogenous energy loss (E_e) for each bird over the entire experimental period (Guillaume and Summers, 1970). Endogenous nitrogen loss, also for the entire experimental period, was estimated using the value 0.1 gN.(kg body mass)$^{-0.75}$.d^{-1} calculated for wild birds (Robbins, 1983). Birds were weighed at the start of each feeding trial, after the initial fast.

MEC_p was calculated for each individual bird in every feeding trial, and these predicted values compared with corresponding observed values (MEC_o) calculated by converting AE_{eN} values from percentages to fractions, and taking endogenous energy losses into account, so that:

$$MEC_o = 1 - (E_{in} - E_{ex} - N - E_e)/E_{in} \quad (5)$$

The values thus obtained may be considered estimates of true rather than apparent MEC, because endogenous losses of both energy and nitrogen are considered. Single pooled faecal samples were collected from each bird for each feeding trial, hence total quantities of energy, nitrogen and refractory material ingested and excreted, rather than values per day, were used for the calculation of both MEC_p and MEC_o. The exact duration (d) of each feeding trial was known. As all terms in Equation (4) are fractions, use of totals rather than daily values did not affect MEC_p estimates.

Statistical methods

Non-parametric statistical tests were used for all inter- and intraspecific comparisons of AE, MEC_o and MEC_p. The Kruskal-Wallis single factor analysis of variance confirmed significant between-group variances, whereafter significantly different species pairs or pairs of food types were isolated with an *a priori* test that

uses rank sums (Dunn, 1964; see also Chapter 3). The two-tailed Wilcoxon U-test was used for single pairwise comparisons between independent sample sets, and the Wilcoxon paired-sample test to detect significant differences between the predicted and observed values of MEC for each food type within each bird species. A sampled randomization test (Sokal and Rohlf, 1969) was used to detect significant differences in variance between predicted and observed values of MEC.

Bird diet

The natural diets of the species studied were inferred from published studies carried out at Marion Island and in the southwestern Cape, South Africa.

RESULTS

Food composition

Ten pilchards, 16 anchovies, 200 g (wet mass) of light-fish and 400 g of squid were used as representative food samples for laboratory analyses. Three separate lots of krill were used in the feeding experiments, and a subsample of minimum mass 200 g was taken from each lot for analyses. The values given in Table 4.1 are means, but separate values for each lot were used in the calculations of AE and MEC_o for birds fed krill. A 300 g subsample of prawn was taken for the analyses.

Energy values of the different food types varied between 17 and 21kJ.g^{-1} dry mass, with crustaceans having lower values than fish or squid (Table 4.1). Crustaceans had the highest ash and calcium content of the three catgories, and squid the lowest. Nitrogen concentrations were similar in all three food categories, and krill had the highest lipid content, followed by fish, prawn and finally squid (Table 4.1).

Meal masses were between 5 and 16% of bird body mass, and most birds lost between 0.6 and 9% of their body mass during the feeding trials (Table 4.2). White-

Table 4.1. Water content (% total wet mass), energy ($kJ.g^{-1}$ dry mass), nitrogen, lipid, calcium and ash content (% dry mass) of foods fed to experimental birds. Standard deviations for krill composition values given in parentheses. n.d.: no data.

Food type	Water	Energy	Nitrogen	Lipid	Calcium	Ash	Chitin
Anchovy *Engraulis japonicus capensis*	71.18	21.78	9.5	7.9	3.1	10.7	n.d.
Light-fish *Maurolicus muelleri*	72.30	20.50	9.9	9.6	3.7	13.2	n.d
Pilchard *Sardinops ocellatus*	72.60	20.04	9.7	7.4	4.5	14.5	n.d.
Squid *Loligo vulgaris reynaudii*	76.44	20.09	9.1	3.5	1.1	6.3	n.d.
Antarctic Krill *Euphausia superba*	79.89 (4.53)	17.87 (1.91)	8.2 (1.1)	11.2 (4.8)	1.8 (0.2)	15.8 (3.6)	2.9 (0.4)
Prawn *Penaeus indicus*	70.67	17.05	8.7	4.1	5.2	16.6	6.5 (0.1)

Table 4.2. Mean meal masses (wet, g) expressed as percentages of bird body masses, and bird body mass changes from the start to the end of the feeding trials (as percentages of fasted mass). MM = meal mass; BM = change in body mass. Figures in parentheses = 1 standard deviation.

Food type	Fish		Squid		Crustacea	
Species	MM	BM	MM	BM	MM	BM
Cape Gannet *Morus capensis*	9.16 (0.44)	-3.92 (2.07)	10.05 (0.76)	-5.63 (3.04)	9.40 (0.83)	-6.21 (2.69)
Blue Petrel *Halobaena caerulea*	10.52 (0.96)	2.09 (2.29)	18.22 (1.50)	-2.62 (6.07)	9.44 (4.26)	-1.61 (4.34)
White-chinned Petrel *Procellaria aequinoctialis*	7.84 (0.34)	1.52 (1.23)	9.84 (1.26)	0.94 (1.01)	9.98 (0.60)	1.30 (0.89)
Sooty Albatross *Phoebetria fusca*	16.03 (0.44)	-0.63 (3.28)	14.57 (0.45)	-9.01 (3.60)	16.78 (0.72)	5.85 (3.68)
Rockhopper Penguin[1] *Eudyptes chrysocome*	7.04 (1.03)	-3.32 (1.79)	4.97 (0.36)	-4.55 (0.87)	5.28 (0.71)	-1.29 (1.72)
Rockhopper Penguin[2]	6.18 (0.58)	-3.17 (1.67)	6.34 (0.50)	-3.23 (2.55)	5.63 (1.05)	-3.97 (1.59)
Gentoo Penguin *Pygoscelis papua*	7.36 (0.68)	-1.21 (0.37)	6.53 (0.47)	-1.85 (0.41)	6.52 (1.03)	-3.28 (1.06)
King Penguin *Aptenodytes patagonicus*	6.37 (0.25)	-2.52 (1.28)	6.81 (0.63)	-0.58 (1.31)	5.74 (0.45)	-2.93 (3.16)

1: Post-moult birds, Marion Island
2: Pre-laying birds, Gough Island

chinned Petrels gained between 0.9 and 1.5% of body mass during the experimental period.

Assimilation efficiencies for each dietary component varied between individuals, species, and between food types (Tables 4.3, 4.4 and 4.5). For all species, values for AE_e ranged from 49.30 to 81.31%, and correction for nitrogen retention most frequently resulted in lower AE_{eN} values (39.99 - 73.94%). Nitrogen was more frequently retained than excreted, with AE_N estimates ranging from -9.99 to 80.34%. Assimilation efficiencies of lipids were generally higher in the flying species (47.52 - 94.75%) than in the penguins (7.17 - 81.54%), and AE_{Ca} varied between -20.48 and 71.55%).

Assimilation efficiency and meal size

King Penguins fed pilchard meals ranging from approximately 350 g to 1000 g (wet mass) showed no significant differences in assimilation efficiencies of any dietary components in relation to meal size, with overall mean values of AE_{eN} equal to 62.72 ± 10.03%. Mean MEC_p (0.733 ± 0.034 did not differ from MEC_o (0.700 ± 0.084) for any of the four meal sizes, nor did actual MEC_o values differ significantly between meal sizes. Experimental birds lost a mean of 2.00 ± 2.37% of their body mass during the experimental period.

The relationship between meal size and MEC_p can be inferred from Fig. 4.1, which shows a regression of MEC_p against a range of simulated meal sizes. Values of MEC_p were calculated for a bird of body mass 11.2kg (the mean body mass of all King Penguins used in this study), fed food with an energetic value of 20.036 $kJ.g^{-1}$, and a nitrogen content of 9.7% (dry mass), both measured values for the pilchard used in this study (Table 4.1). As in the calculations of MEC_o for pilchard (see Methods section), the food was assumed to contain no material refractory to chemical digestion.

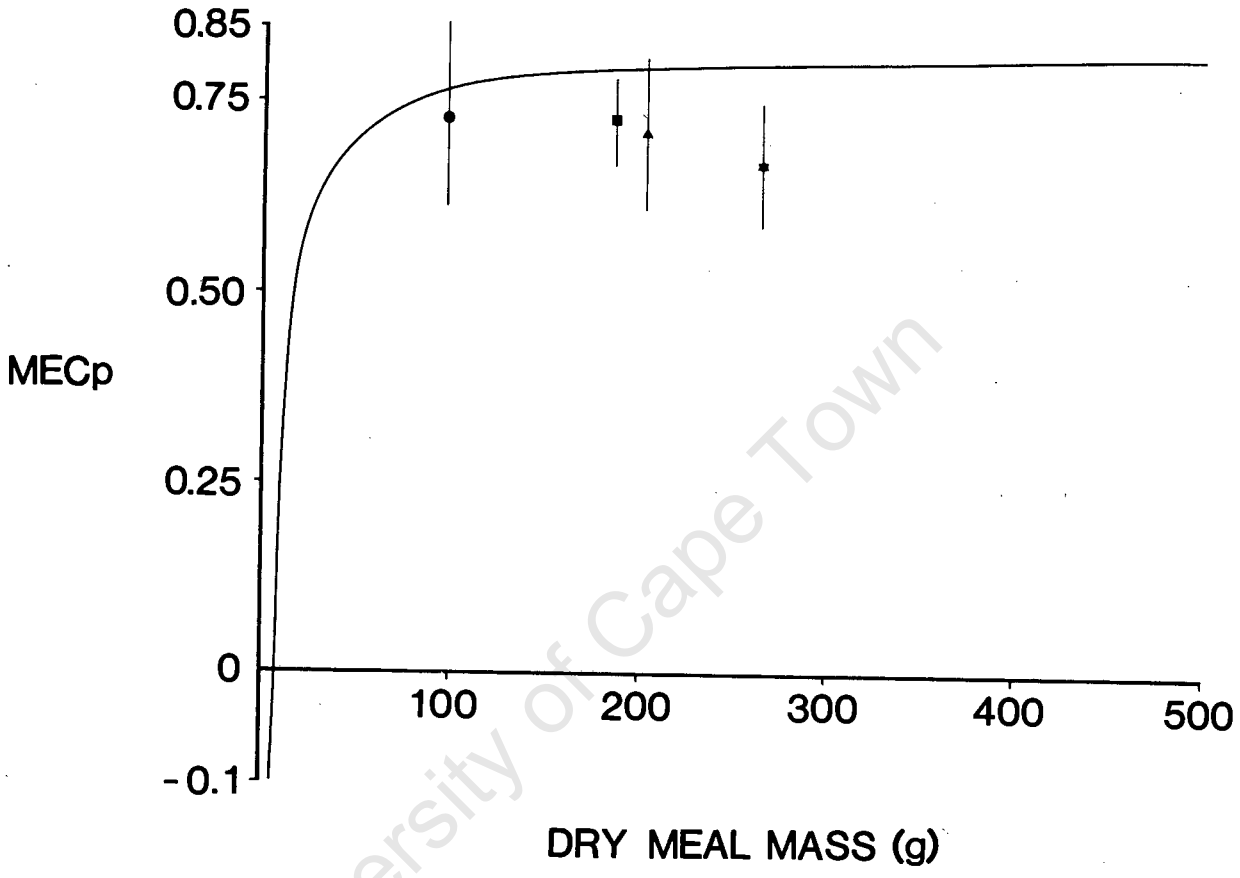

Figure 4.1. MEC_p for pilchard plotted against meal size, and observed MEC's of pilchard fed to King Penguins *Aptenodytes patagonicus*.
350 g meal: ● , 300 g meal x 2: ■ , 750 g meal: ▲ , 1000 g meal: ★ .
Note: MEC_p values for each meal-size group do not fall exactly on the predicted curve, because the regression was calculated using an overall mean body mass for the species.

Table 4.3. Mean energy assimilation efficiencies (AE_e), energy assimilation efficiencies corrected for nitrogen retention (AE_{eN}), metabolizable energy coefficients (MEC_o), and lipid (AE_{lip}), calcium (AE_{Ca}) and nitrogen (AE_N) assimilation efficiencies for seven species of seabird fed fish. N = sample size. Figures in parentheses = 1 SD.

Species	N	AE_e	AE_{eN}	[1]MEC_o x 100	AE_{lip}	AE_{Ca}	AE_N
1. Cape Gannet *Morus capensis*	5	78.30 (3.30)	70.90 (2.45)	75.53 (1.48)	84.31 (4.11)	45.18 (9.57)	38.59 (18.54)
2. Blue Petrel *Halobaena caerulea*	6	72.52 (7.01)	72.88 (2.60)	77.42 (2.17)	82.50 (2.51)	-20.48 (32.33)	-2.30 (28.20)
3. White-chinned Petrel *Procellaria aequinoctialis*	6	78.17 (1.64)	69.78 (2.51)	75.61 (2.03)	87.75 (2.69)	31.79 (30.58)	42.07 (7.32)
4. Sooty Albatross *Phoebetria fusca*	5	75.61 (1.44)	72.59 (1.67)	76.51 (1.59)	78.24 (1.82)	-47.40 (17.41)	18.93 (7.65)
5. Rockhopper Penguin *Eudyptes chrysocome*	6	65.51 (5.19)	58.97 (5.48)	75.13 (5.81)	27.30 (19.01)	28.40 (16.44)	37.03 (10.97)
6. Gentoo Penguin *Pygoscelis papua*	6	79.09 (4.64)	70.15 (3.92)	78.08 (3.49)	81.54 (12.44)	21.74 (16.87)	50.59 (14.17)
7. King Penguin *Aptenodytes patagonicus*	6	68.76 (10.99)	61.12 (9.91)	70.89 (9.99)	40.78 (37.83)	46.17 (25.05)	43.23 (8.30)
Significant differences between species pairs		3 *v* 5* 5 *v* 6*	2 *v* 5** 4 *v* 5**		3 *v* 5** 3 *v* 7*; 5 *v* 6*	1 *v* 4** 4 *v* 7**	2 *v* 3* 2 *v* 6**; 2 *v* 7*
Mean for all species		73.99 (5.26)	68.06 (5.63)	75.60 (2.33)	68.92 (24.31)	15.06 (35.45)	32.59 (18.20)

[1] Values for MEC_O are expressed as percentages rather than fractions to facilitate comparison with AE values.
Significantly different species pairs within each food type denoted by asterisks: *: $P < 0.05$; **: $P < 0.01$.

Table 4.4. Mean AE_e, AE_{eN}, MEC_o, AE_{lip}, AE_{Ca} and AE_N estimates for seven species of seabird fed squid. All figures are expressed as percentages of the original quantities ingested. Abbreviations as for Table 4.3.

Species	N	AE_e	AE_{eN}	MEC_o x 100	AE_{lip}	AE_{Ca}	AE_N
1. Cape Gannet *Morus capensis*	5	69.56 (2.98)	67.79 (2.85)	72.18 (3.06)	47.52 (20.58)	42.65 (17.78)	12.52 (13.71)
2. Blue Petrel *Halobaena caerulea*	6	63.60 (2.38)	63.78 (2.22)	68.32 (2.30)	51.74 (22.11)	65.81 (6.81)	-0.98 (5.42)
3. White-chinned Petrel *Procellaria aequinoctialis*	7	74.61 (1.83)	67.65 (2.56)	76.58 (1.93)	56.99 (10.30)	71.55 (9.25)	31.71 (6.79)
4. Sooty Albatross *Phoebetria fusca*	6	67.18 (1.36)	69.06 (1.25)	75.62 (1.31)	49.85 (5.46)	43.04 (10.55)	-9.99 (6.18)
5. Rockhopper Penguin *Eudyptes chrysocome*	6	74.90 (5.22)	66.11 (5.36)	76.98 (4.78)	7.17 (8.56)	29.40 (30.12)	65.02 (9.17)
6. Gentoo Penguin *Pygoscelis papua*	6	69.54 (4.42)	67.01 (4.33)	76.44 (4.61)	59.46 (5.22)	26.52 (37.21)	17.49 (19.18)
7. King Penguin *Aptenodytes patagonicus*	5	76.28 (1.70)	69.88 (1.53)	79.88 (1.87)	51.11 (10.94)	63.15 (14.31)	44.11 (8.68)
Significant differences between species pairs		2 *v* 3** 2 *v* 5**; 2 *v* 7**			3 *v* 5*	3 *v* 5*	2 *v* 5*; 3 *v* 4* 4 *v* 5**; 4 *v* 7**
Mean for all species		71.45 (5.53)	67.33 (2.00)	75.14 (3.77)	46.26 (17.73)	48.87 (18.06)	22.84 (26.11)

Table 4.5. Mean AE_e, AE_{eN}, MEC_o, AE_{lip}, AE_{Ca} and AE_N estimates for seven species of seabird fed crustacea. All figures are expressed as percentages of the original quantities ingested. "in.s." = insufficient sample for analysis. Other abbreviations as for Table 4.3.

Species	N	AE_e	AE_{eN}	MEC_o x 100	AE_{lip}	AE_{Ca}	AE_N
1. Cape Gannet *Morus capensis*	6	65.66 (8.03)	60.13 (5.22)	70.78 (3.48)	60.64 (12.88)	45.87 (9.87)	27.28 (29.44)
2. Blue Petrel *Halobaena caerulea*	6	71.43 (9.41)	67.82 (6.16)	74.54 (7.18)	87.69 (4.58)	in.s.	26.73 (25.44)
3. White-chinned Petrel *Procellaria aequinoctialis*	5	76.16 (2.14)	67.52 (1.65)	75.79 (1.69)	94.75 (2.18)	in.s. (4.98)	42.04
4. Sooty Albatross *Phoebetria fusca*	6	76.03 (3.66)	73.94 (2.87)	78.43 (2.87)	88.25 (3.92)	in.s.	15.46 (5.99)
5. Rockhopper Penguin *Eudyptes chrysocome*	5	70.02 (5.19)	58.69 (4.53)	(79.31) (4.72)	43.61 (21.17)	51.77 (10.76)	62.79 (8.57)
6. Gentoo Penguin *Pygoscelis papua*	3	77.44 (8.94)	67.50 (6.80)	78.20 (4.76)	11.38 (61.27)	22.73	55.57 (13.52)
7. King Penguin *Aptenodytes patagonicus*	2	63.25	60.75	72.89	25.43	32.96	13.34
Significant differences between species pairs		3 *v* 7* 4 *v* 7*	1 *v* 4* 4 *v* 5**; 5 *v* 7*		3 *v* 7**		4 *v* 5*
Mean for all species		71.43 (5.51)	65.19 (5.50)	75.71 (3.17)	58.82 (33.14)	38.33 (13.03)	34.75 (19.25)

Interspecific differences in assimilation efficiency

All differences listed below are statistically significant. Values of the Kruskal-Wallis test statistic H, and of Y_m/S_m, and significance levels for every pairwise comparison, are listed in Appendix 4.1.

(a) Energy assimilation

Among birds fed fish (see Table 4.3), energy assimilation efficiencies were higher in both White-chinned Petrels and Gentoo Penguins than in Rockhopper Penguins. Among birds fed squid (Table 4.4), White-chinned Petrels, Rockhopper and King Penguins all showed higher AE_e's than did Blue Petrels. Energy assimilation efficiencies among birds fed crustaceans (Table 4.5) were higher in both White-chinned Petrels and Sooty Albatrosses than in King Penguins.

(b) Nitrogen-corrected energy assimilation

Among birds fed fish, AE_{eN} values for both Blue Petrels and Sooty Albatrosses were higher than those for Rockhopper Penguins. There were no significant interspecific differences between AE_{eN}'s for birds fed squid, and AE_N values for this food category were higher for Sooty Albatrosses than for Cape Gannets and Rockhopper and King Penguins.

(c) Lipid assimilation

Lipid assimilation efficiencies were generally highest amongst birds fed fish. When fed this food type, White-chinned Petrels assimilated lipids more efficiently than did both Rockhopper and King Penguins, and Gentoo Penguins did so more efficiently than did Rockhopper Penguins. Squid-fed White-chinned Petrels assimilated lipids more efficiently than did Rockhopper Penguins, and amongst birds fed crustaceans, White-chinned Petrels assimilated lipids more efficiently than did King Penguins.

(d) Calcium assimilation

Amongst birds fed fish, calcium assimilation efficiencies were higher in both Cape Gannets and King Penguins than in Sooty Albatrosses, and White-chinned Petrels fed squid assimilated calcium more efficiently than did Rockhopper

Penguins. Rockhopper Penguins fed crustaceans assimilated calcium more efficiently than did King Penguins.

(e) Nitrogen assimilation

Amongst birds fed fish, White-chinned Petrels, Gentoo and King Penguins all assimilated nitrogen more efficiently than did Blue Petrels. For squid-fed birds, nitrogen assimilation efficiencies differed between four species pairs: White-chinned Petrels and Rockhopper and King Penguins all exhibited higher values than did Sooty Albatrosses, and Rockhopper Penguins higher values than Blue Petrels. Rockhopper Penguins assimilated nitrogen more efficiently than did Sooty Albatrosses when fed crustaceans.

When all food types were considered together, interspecific differences in assimilation efficiencies of the different dietary components were predominantly between procellariiforms and penguins (5 of a total of 7 for AE_e, 3/4 for AE_{eN}, 6/7 for AE_N, 4/5 for AE_{lip} and 2/3 for AE_{Ca}). There were no significant differences in AE between the gannet and any of the three penguin species, and interspecific differences within procellariiforms and within penguins were few (two in each case).

Intraspecific differences between assimilation efficiencies of different foods

Comparison of assimilation efficiencies between food types revealed significant differences within each species (Fig. 4.2, see Appendix 4.2 for all statistical values).

For the Cape Gannet, both AE_e and AE_{eN} were higher in birds fed fish than in birds fed crustaceans. This species also assimilated lipids more efficiently when fed fish than when fed squid. Values of AE_{eN} for the Blue Petrel were significantly higher in birds fed fish than in those fed squid, and Blue Petrels assimilated lipids and nitrogen more efficiently when fed crustaceans than when fed squid. For the White-chinned Petrel, AE_e and AE_N were higher in birds fed light-fish than in birds fed squid. Birds fed crustaceans assimilated lipids more efficiently than did squid-fed birds, whereas birds fed squid assimilated calcium more efficiently than did those fed fish. AE_e and AE_N values were higher in Sooty Albatrosses fed both fish

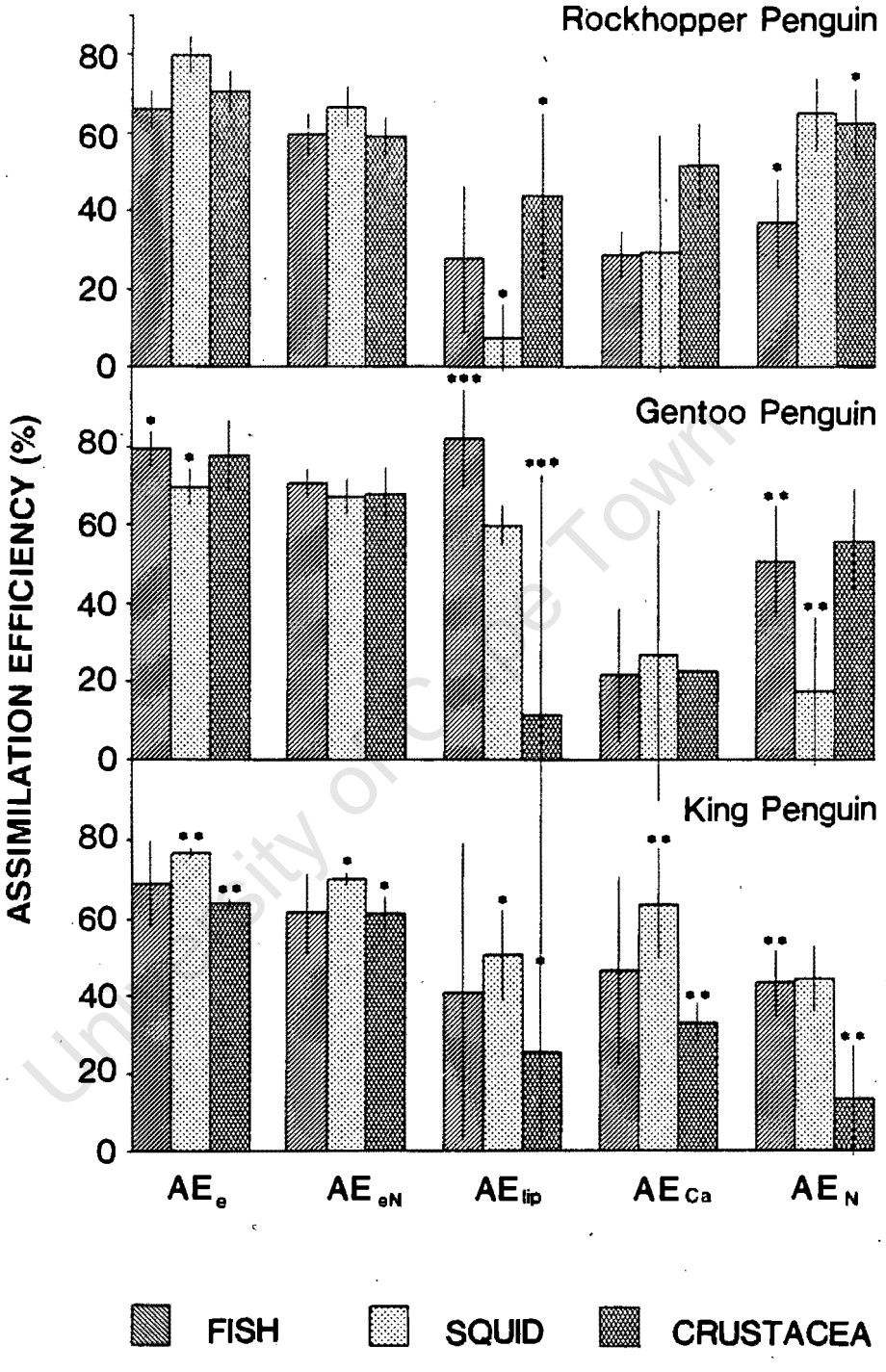

Figure 4.2. Assimilation efficiencies (AE) of different dietary components in seven seabird species fed fish (F), squid (S) and crustaceans (C). Abbreviations for AE explained in the text, and in Table 4.3. W-c Petrel: White-chinned Petrel

* $P < 0.05$, ** $P < 0.025$, *** $P < 0.01$.

and crustaceans, than in birds fed squid. AE_{eN} and AE_{lip} were higher in crustacea-fed than in squid-fed albatrosses.

Rockhopper Penguins assimilated lipid more efficiently when fed crustaceans than when fed squid, and AE_N was higher in birds on a crustacean diet than in birds fed fish. Gentoo Penguin AE_e and AE_N were significantly higher in fish-fed birds than in birds fed squid. Gentoo Penguins assimilated lipids more efficiently when fed fish than when fed crustaceans. Assimilation efficiencies of energy (both AE_e and AE_{eN}), lipid and calcium were higher in King Penguins fed squid than in birds fed crustaceans, and assimilation of nitrogen was more efficient in birds fed fish than in those fed crustaceans.

A separate pairwise comparison showed that post-moulting Rockhopper Penguins fed prawns had significantly higher AE_e, AE_{eN} and AE_N than did their conspecifics fed krill (Table 4.6, in all cases $U_{3,6} = 18$, $P < 0.025$).

Interspecific differences between metabolizable energy coefficients of different foods

In the Cape Gannet, both MEC_p and MEC_o of fish were significantly greater than corresponding coefficients of crustaceans (all Kruskal-Wallis test values given in Appendix 4.3). MEC_p and MEC_o of fish fed to Blue Petrels were significantly greater than corresponding values of squid. Among White-chinned Petrels, MEC_p's of fish were greater than of squid, but MEC_o values did not differ between food types. The same is true for Sooty Albatrosses.

Among Rockhopper Penguins, MEC_p of squid was significantly higher than corresponding crustacean values, but MEC_o's of all foods were statistically indistinguishable. For Gentoo Penguins, predicted MEC's of fish were higher than those of crustaceans, but no difference was detectable between MEC_o values. The same is true for King Penguins.

Correlation between MEC_p and MEC_o

Predicted estimates of MEC were almost all within 10% of the observed values (Fig. 4.3). However, MEC_o was significantly correlated with MEC_p for only the

Table 4.6. Mean estimates of AE_e, AE_{eN}, MEC_o, AE_{lip}, AE_{Ca} and AE_N for pre-laying (PL) and post-moulting (PM) Rockhopper Penguins *Eudyptes chrysocome* fed fish, squid and crustacea. All figures are expressed as percentages of the original quantities ingested. Asterisks and vertical lines to the right of values denote significant differences between pre-laying and post-moult birds, whereas asterisks and vertical lines to the left of values denote significant differences between krill- and prawn-fed post-moult birds. *: $P \leq 0.05$, **: $P \leq 0.01$.

Food type and bird condition		N	AE_e	AE_{eN}	MEC_o x 100	AE_{lip}	AE_{Ca}	AE_N
Pilchard	PL	6	65.51 (5.19)	58.97** (5.48)	75.13 (5.81)	27.30 (19.01)	28.40** (16.44)	37.03 (10.97)
	PM	6	53.49 (23.55)	39.99** (20.42)	57.55 (19.79)	in.s. (25.43)	82.40** (18.16)	51.39
Squid	PL	6	74.90* (5.22)	66.11 (5.36)	76.98** (4.78)	7.17 (8.56)	29.40 (30.12)	65.02** (9.17)
	PM	6	81.31* (4.77)	69.83 (4.10)	87.43** (5.71)	in.s.	50.48 (14.24)	79.15** (5.45)
Prawn	PL	5	70.02 (5.19)	58.69 (4.53)	79.31 (4.72)	43.61** (21.17)	51.77* (10.76)	62.79** (8.57)
	PM	3	**72.59 (5.62)	**58.22 (5.36)	**71.67 (6.29)	0.84** (1.45)	23.72* (18.91)	**80.34** (5.37)
Krill	PM	6	**49.30 (7.00)	**44.20 (5.16)	**82.53 (7.30)	68.76 (20.12)	in.s.	**27.90 (21.73)

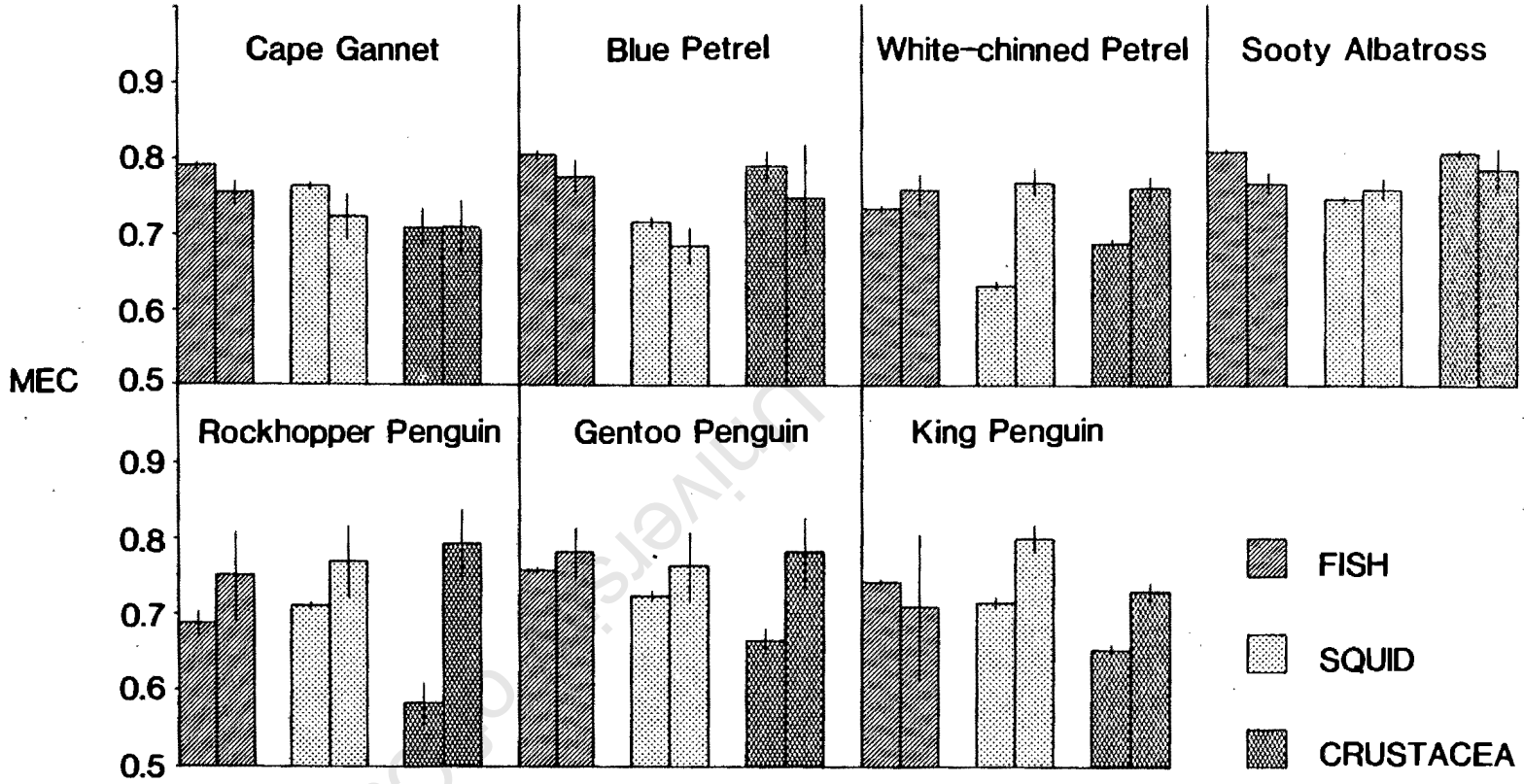

Figure 4.3. Predicted (MEC_p) and observed (MEC_o) metabolizable energy coefficients (MEC) of fish, squid and crustaceans fed to seven seabird species. Of each pair of bars, that on the left represents MEC_p, and that on the right MEC_o. Significant differences between MEC_p and MEC_o denoted by asterisks. *: $P < 0.05$, **: $P < 0.01$.

Cape Gannet ($y = 0.573x + 0.298$, $r = 0.68$, $P < 0.0025$ for 14 df, where $MEC_o = y$ and $MEC_p = x$)) and the Blue Petrel ($y = 0.766x + 0.146$, $r = 0.55$, $P < 0.01$ for 16 df). With the exception of Blue Petrels and post-moult Rockhoppers fed crustaceans, MEC_o values varied significantly more within food types for each species than did MEC_p's (sampled randomization tests, in all cases $P \leq 0.01$).

Correlation between AE_e and MEC_o, and mean retention time

For Cape Gannets and the three penguin species, there was no significant relationship between either AE_e or MEC_o of each of the three food types, and mean retention times of that food in the gut (Chapter 3). This is unexpected, all the more so because the AE and MEC values and the mean retention times were all calculated using data collected simultaneously from each bird. MEC_o was not significantly correlated with hindgut area for any food type.

Assimilation efficiency in relation to moult and maturity

Pre-laying Rockhopper Penguins fed squid exhibited lower AE_N values than did post-moult birds on the same diet (Table 4.6, $U_{5,6} = 28$, $P \leq 0.02$). However, AE_{eN} was higher in pre-laying birds on a fish diet than in post-moulting birds fed the same food ($U_{5,6} = 27$, $P \leq 0.05$). Amongst birds fed fish, post-moult Rockhopper Penguins assimilated calcium more efficiently than did pre-laying individuals ($U_{6,6} = 34$, $P \leq 0.01$). Finally, pre-laying Rockhopper Penguins fed crustaceans assimilated lipids and calcium more efficiently than did post-moult conspecifics ($U_{3,5} = 15$, $P \leq 0.05$ in both cases).

Sooty Albatross fledglings assimilated the calcium and nitrogen in krill meals significantly more efficiently than did adults of this species (Table 4.7; $U_{5,6} = 30$, $P < 0.005$; $U_{5,6} = 28$, $P \leq 0.02$ respectively).

DISCUSSION

The composition of foods used in this study is similar to published values for fish and pilchard (Simmonds and Turner, 1980), squid (Cooper, 1979) and krill

Table 4.7. Mean AE_e, AE_{eN}, MEC_o, AE_{lip}, AE_{Ca} and AE_N estimates for Sooty Albatross *Phoebetria fusca* adults and fledglings fed krill. All figures are expressed as percentages of the original quantities ingested. Abbreviations as for Table 4.3.

	N	AE_e	AE_{eN}	MEC_o x 100	AE_{lip}	AE_{Ca}	AE_N
Adults	6	66.39 (2.49)	66.50 (3.63)	75.16 (3.52)	92.49 (1.09)	-78.76** (14.59)	-0.65** (20.83)
Fledglings	6	73.38 (7.23)	67.37 (4.28)	76.87 (4.72)	90.35 (5.86)	22.85** (16.78)	34.97** (21.38)

(Clarke, 1980). Prawn has a higher chitin content than krill and is consequently significantly less digestible *in vitro* (Chapter 1), and not an ideal representative crustacean. Significantly higher assimilation efficiencies among post-moulting prawn-fed rockhoppers than among birds fed krill may be an artefact resulting from retention of undigested prawn. This unfortunately confounds interpretation of intraspecific comparisons between this and other food types.

Meal mass and assimilation efficiency

Miller and Reinecke (1984) caution that apparent and true metabolizable energy of avian foods should be measured at feeding levels high enough for maintenance of body mass. Karasov (in press) states that this may be unneccessarily rigorous. The present study supports his view, because of the lack of significant differences in AE's and MEC_o's of any dietary components between King Penguins fed pilchard meals of different sizes. Fig. 4.1 indicates that meal mass exerts the greatest influence on MEC_p when meal masses are small, and endogenous energy and nitrogen losses are consequently high in relation to total faecal quantities. For meals of dry mass over 1.8% of bird body mass (for pilchard, this is equivalent to a wet meal mass approximately 6.5% of bird body mass), MEC_p differs little over a wide range of meal sizes. The critical lower limit for meal size may be a more important factor for consideration in experimental design than the feeding level required for maintenance, particularly in studies using brief feeding trials, or species that cannot be induced to feed *ad libitum*.

Although the experimental birds could not be induced to take a range of meal sizes wide enough to yield a statistically-significant trend, actual MEC's appear to decrease with increasing meal size in a manner not predicted by Karasov's model. In the absence of compensatory slowing of gut passage rates, absorption of nutrients is probably less efficient for large meals because proportionally less digesta come into contact with the absorptive surfaces of the gut per unit time. Although initial gut retention time of the small pilchard meals was less than that of larger meals (Chapter 3), overall mean retention times for different meal sizes did not differ

significantly. Seabird gut passage rates thus do not appear to slow in response to larger meal sizes, although this may not be true of birds feeding chicks (Wilson *et al.*, subm.).

Assimilation efficiency in relation to natural diet

Of the seabirds studied, the Cape Gannet is the most specialized piscivore. In southwestern Cape waters, pelagic shoaling fish constitute 61% of its diet by mass, hake scavenged from demersal trawlers 32%, and other prey, including cephalopods, 7%. Crustaceans are a negligible portion of the diet by mass (Berruti *et al.*, 1989). At Marion Island, King Penguins too prey primarily on fish (87% of the diet by mass) and squid (13%), with crustaceans contributing less than 1% to the diet by mass (Adams and Brown, 1989). Gentoo Penguins eat a mixture of fish (53% of the diet by mass) and crustaceans (44%), with squid contributing 2% to the diet by mass (Adams and Brown, 1989). White-chinned Petrels wintering in the southern Benguela region are also primarily piscivorous, with meso-pelagic and pelagic fish constituting 72% of the diet by mass, crustaceans 13% and squid 11% (Jackson, 1988). Squid are, however, an important component of the diet of this species in the breeding season at Marion Island (A. Berruti, *pers. comm.*) and off New Zealand (Imber, 1976). White-chinned Petrels at South Georgia prey on similar quantities of squid (47%), fish (24%) and crustacea (30%)(Croxall and Prince, 1980). Sooty Albatrosses prey mainly on squid at the Crozet Islands (Mougin, 1970), and eat squid at Marion Island (Berruti and Harcus, 1978). The latter study provided no information on the importance of squid relative to other prey, however, as it was based purely on analyses of squid beaks.

Crustaceans are the major prey (60% by mass) of Blue Petrels, with squid contributing 15% and fish 21% to their diet by mass (Steele and Klages, 1986). The same is true of Rockhopper Penguins: crustaceans constitute 81% of the diet by mass, fish 15% and squid 3% (Adams and Brown, 1989).

Intraspecific variation in energy assimilation efficiencies of different food types showed no clear trend reflecting dietary specialization, as each species did not

appear to assimilate its major natural prey type more efficiently than it did less frequently eaten prey. It is significant that values of MEC_o showed fewer inter- and intraspecific differences for the three food types than did corresponding AE_{eN} values, because MEC values are independent of both the length of the feeding experiments and of bird body mass. Differences between MEC_p and MEC_o within prey types should therefore reflect dietary adaptations (Karasov, in press), whereas differences in AE_{eN} may simply reflect differences in feeding trial duration, and in bird body mass, which differed substantially both inter- and intraspecifically.

MEC_o's of squid for White-chinned Petrels were significantly higher than predicted values. Squid is important in the diet of this species, but the same can be said of Sooty Albatrosses, and of King Penguins at South Georgia (Croxall and Prince, 1980). This sole difference between MEC_o and MEC_p cannot realistically be interpreted as digestive specialization by White-chinned Petrels.

Comparison of variability within these two parameters is instructive: within each species and each food type, MEC_o values show significantly higher variances than do MEC_p's. This presumably accounts for the greater number of statistically significant inter- and intraspecific differences amongst the latter. Karasov's model (Equation (4)) was intended primarily as an heuristic exercise, and its use here has demonstrated that MEC is not predictable solely on the basis of prey composition and mass-specific metabolic parameters. Experimenters using a comparative approach should take variance into account when determining sample sizes: Moss (1983) found that grouse digestive efficiencies varied as much intraspecifically as interspecifically. Refinement of Karasov's model and of MEC_o estimates by incorporation of experimentally-determined E_e and E_N values specific to seabirds would increase the predictive value of the model, but similar refinement would not reduce the degree of inter-individual variation in MEC_o values calculated using Equation (5).

The lack of visible trends might indicate that the prey types used here are too similar for their composition to be reflected in digestive specialization, but

significant differences between digestibilities of these foods *in vitro* disprove this. Alternatively, assimilation efficiency may be adaptive precisely *because* it is not directly influenced by prey composition. This conclusion is supported by the similarity of MEC_0's for food types which contain widely different structural compounds such as the collagen in squid muscle (Bone *et al.*, 1981) and the chitin in crustacean exoskeleton. The observed metabolizable energy coefficients presented here differ from those observed for birds at other trophic levels such as herbivores (Karasov, in press), but may be physiologically and energetically optimal for marine predatory birds.

Energy balance: implications for energetics studies

Assumed energy assimilation (or "utilization") efficiencies in models of seabird energetics and food consumption have varied from 70% (Wiens and Scott, 1975), to 75% (Furness, 1978) or 80% (Berruti *et al.*, 1985). Schneider and Hunt (1982) and Guillet and Furness (1985) have both cited Kendeigh *et al.* (1977) as their source for two different estimates of a general metabolizable energy coefficient for avian food (70 and 80%, respectively); Kendeigh *et al.* (1977), in fact, review a range of published MEC values from 39 to 91% for passerines and non-passerines fed various food types.

Measured apparent energy AE's in seabirds are similar to the values assumed above: mean measured AE's were 81.9% for nestling Double-crested Cormorants fed pollack *Pollachius virens* (Dunn, 1975), 75.3% for growing Jackass Penguins *Spheniscus demersus* chicks fed fish (Cooper, 1977), 74.5% for Cape Gannet chicks and juveniles fed fish (Cooper, 1978). Assimilation efficiencies of four King Penguins fed squid averaged 81.3% (Adams, 1984). None of these values were corrected for nitrogen retention, or for endogenous nitrogen and energy losses, and they are thus comparable to AE_e values in the present study. Davis *et al.* (1989) report a metabolizable energy coefficient (corrected for fecal and urinary nitrogen losses) of 0.74 for Gentoo Penguins fed krill.

Across all seabird species used in the present study, mean values of AE_e, AE_{eN} and MEC_o were similar for all three food types. Correction for endogenous nitrogen and energy losses yields MEC_o values that, expressed as percentages, are most frequently higher than AE_e estimates by between one and ten percent. Except for the study of Davis *et al.* (1989), all previously published estimates of assimilation efficiency in seabirds have been uncorrected for either nitrogen retention, or for endogenous losses of nitrogen and energy. In energetics studies and consumption models, the best assessment of a bird's energy requirements will theoretically be made using true MEC values corrected for such endogenous losses. In view of the relatively minor differences between AE_e and MEC_o estimates calculated in the present study, the former values probably yield an acceptable degree of accuracy for use in energetics models. The value of 80% used by Berruti *et al.* (1985) for White-chinned Petrels is too high. Confirmation of the accuracy of assumed values should, however, be obtained by experimental determination of endogenous energy losses from seabird guts during digestion (see Miller and Reinecke, 1984, and Karasov, in press), because available data are at present restricted to domestic geese and chickens. True MEC values are more likely to differ from apparent assimilation efficiencies in seabirds with longer guts (which offer a greater surface area for abrasion and cell sloughing), and in birds with low body mass fed at intake levels well below maintenance. For the latter, endogenous losses will be higher in proportion to total faecal energy and nitrogen content because the losses scale with $(\text{body mass})^{0.75}$, as does avian metabolic rate. The duration of feeding trials also influences AE estimates, which have previously been made over a variety of experimental periods, from one (Cooper, 1977, 1978; Dunn, 1975) to five (Adams, 1984) days, and cognisance should be taken of this fact.

Lipid assimilation efficiency

Mean AE_{lip} was highest in birds fed pilchard, followed by crustaceans, and then squid. The relatively low assimilation efficiencies of lipids in squid-fed birds may be a consequence of the low lipid content of this food. The low values of AE_{lip} in

lizards, found that gut surface areas amplified by villi enabled the rodents to assimilate their food more efficiently than did the lizards, despite more rapid passage rates in the rodents. Differences in micro-anatomy of seabird guts are undoubtedly far less marked than differences between vertebrate classes, but Karasov and Diamond's findings illustrate the need for gut measurements on a finer scale than those presented in Chapter 3. Gut dimensions scale with seabird body mass (Chapter 3), and this relationship must influence metabolizable energy coefficients. Detailed measurements of the absorptive surface area of seabird guts are necessary for a meaningful investigation of the extent of this influence.

Maturity, breeding status and assimilation efficiency

Juvenile jackass Penguins *Spheniscus demersus* fed fish, and black-headed gulls *Larus ridibundus* fed earthworms, assimilated their food with increasing efficiency as they matured (Cooper, 1977; Heath and Randall, 1985; Nelsen and Brandl, 1988). The same is true of Double-crested Cormorants *Phalacrocorax auritus* (Dunn, 1975) and Black-bellied Tree Ducks (*Dendrocygna autumnalis*) (Cain, 1976). Immature birds probably have less efficient guts, hence lower absorption efficiencies (Karasov, in press). However, energy assimilation efficiencies and MEC_o's did not differ significantly between the two age groups of Sooty Albatrosses in this study, perhaps because the juveniles used were close to fledging and had fully formed guts. Cooper (1978) reported higher assimilation efficiencies for Cape Gannet chicks (76.1%) than for juveniles (74.2%). Fledglings assimilated calcium and nitogen significantly more efficiently than did adults, presumably because of demands for bone and feather growth.

Avian energy requirements fluctuate in accordance with annual moult and migration cycles, and food assimilation efficiency might be expected to increase during periods of high energy demand. Such an increase has been demonstrated during the pre-migratory fattening period of the Garden Warbler *Sylvia borin* (Bairlein, 1985). Moult is a period of considerable energetic stress in penguins (Groscolas, 1978), which do not feed for the two to four weeks needed for complete

feather renewal. Rockhopper Penguins lose 43% of their body mass over the moult period (Brown, 1985), and presumably must regain this as quickly as possible once they return to sea, replacing lost fat to conserve body heat and in readiness for the next breeding season. Post-moult birds might be expected to assimilate food more efficiently than pre-breeding birds, which are under less energetic stress: moulting Rockhopper Penguins at Marion Island exhibited metabolic rates 1.32 times the resting value (Brown, 1985), whereas egg production in the *Eudyptes* penguins probably does not contribute as much as 10% to their daily energy expenditure (Brown, 1989, using data from Grau, 1982 for *Eudyptes pachyrhynchus*).

In the present study (disregarding comparisons involving prawn, for reasons given at the beginning of the discussion), post-moulting Rockhopper Penguins showed higher assimilation efficiencies of both calcium and nitrogen than did their pre-laying conspecifics, and, in one case (squid-fed birds), higher MEC_o's. The difference in nitrogen assimilation probably reflects increased protein absorption. Although AE_{eN} values for pilchard were higher in pre-laying than for post-moult birds, this must have resulted from a difference in mass-specific endogenous energy losses, because MEC_o's for this food showed no significant difference. Assimilation of certain dietary components appears to be higher in birds needing to make up an nutritional deficit. The elevated MEC_o (87%) of squid in the post-moult birds may be a temporary response to energetic stress.

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APPENDIX 4.1. Values of Kruskal-Wallace test statistics for interspecific pairwise comparisoncomparisons between assimilation efficiencies of birds fed fish, squid and crustaceans. > indicates which of the two species exhibited a higher mean AE value. Names are abbreviated for clarity.

	Food type	Species pair	Degrees of freedom	H	P <	Y_m/S_m	P <
AE_c							
	FISH	White-chin > Rockhopper	6	21.469	0.005	3.432	0.05
		Gentoo > Rockhopper	"	"	"	3.581	0.05
	SQUID	White-chin > Blue Petrel	6	27.257	0.001	3.665	0.01
		Rockhopper > Blue Petrel	"	"	"	3.687	0.01
		King > Blue Petrel	"	"	"	3.998	0.01
	CRUSTACEA	White-chin > King	6	18.976	0.005	3.212	0.05
		Sooty > King	"	"	"	3.302	0.05
AE_{cN}							
	FISH	Blue Petrel > Rockhopper	6	22.980	0.001	3.679	0.01
		Sooty > Rockhopper	"	"	"	3.390	0.05
	CRUSTACEA	Sooty > Gannet	6	23.068	0.001	3.480	0.05
		Sooty > Rockhopper	"	"	"	3.642	0.01
		Sooty > King	"	"	"	3.454	0.05
AE_{lip}							
	FISH	White-chin > Rockhopper	6	26.646	0.001	4.025	0.01
		White-chin > King	"	"	"	3.284	0.05
		Gentoo > Rockhopper	"	"	"	3.260	0.05
	SQUID	White-chin > Rockhopper	6	14.074	0.05	3.153	0.05
	CRUSTACEA	White-chin > King	6	28.853	0.001	4.197	0.01
AE_{Ca}							
	FISH	Gannet > Sooty	6	21.369	0.005	3.652	0.01
		King > Sooty	"	"	"	3.861	0.01
	SQUID	White-chin > Rockhopper	6	18.502	0.01	3.008	0.05
	CRUSTACEA	Rockhopper > King	4	12.582	0.025	2.726	0.05
AE_N							
	FISH	White-chin > Blue Petrel	6	23.854	0.001	3.235	0.05
		Gentoo > Blue Petrel	"	"	"	3.902	0.01
		King > Blue Petrel	"	"	"	3.358	0.05
	SQUID	Rockhopper > Blue Petrel	6	30.382	0.001	3.348	0.05
		White-chin > Sooty	"	"	"	3.463	0.05
		Rockhopper > Sooty	"	"	"	4.219	0.01
		King > Sooty	"	"	"	3.965	0.01
	CRUSTACEA	Rockhopper > Sooty	6	18.941	0.005	3.302	0.05

APPENDIX 4.2. Values of Kruskal-Wallace test statistics for intraspecific pairwise comparisons of AE's between different food types. > indicates which of the two food types resulted in a higher mean AE value. In all cases, df = 2.

Species		Food types	H	P <	Y_m/S_m	P <
Cape Gannet	AE_e	Fish > Crustacea	9.965	0.025	3.006	0.05
	AE_{eN}	Fish > Crustacea	10.816	0.005	3.249	0.01
	AE_{lip}	Fish > Squid	9.420	0.01	2.758	0.05
Blue Petrel	AE_{eN}	Fish > Squid	8.222	0.025	2.866	0.05
	AE_{lip}	Crustacea > Squid	7.626	0.025	2.7037	0.05
	AE_N	Crustacea > Squid	6.877	0.05	2.596	0.05
White-chinned Petrel	AE_e	Fish > Squid	7.214	0.05	2.686	0.05
	AE_{lip}	Crustacea > Squid	15.053	0.001	3.839	0.01
	AE_{Ca}	Squid > Fish		U7,6 = 35		0.05
	AE_N	Fish > Squid	6.690	0.05	2.325	0.05
Sooty Albatross	AE_e	Fish > Squid	11.058	0.005	2.649	0.05
		Crustacea > Squid	"	"	3.030	0.05
	AE_{eN}	Crustacea > Squid	8.746	0.025	2.801	0.05
	AE_{lip}	Crustacea > Squid	14.235	0.001	3.773	0.01
	AE_N	Fish > Squid	11.129	0.005	2.976	0.05
		Crustacea > Squid	"	"	2.744	0.05
Rockhopper Penguin	AE_{lip}	Crustacea > Squid	6.473	0.05	2.533	0.05
	AE_N	Crustacea > Fish	6.665	0.05	2.474	0.05
Gentoo Penguin	AE_e	Fish > Squid	6.350	0.05	2.453	0.05
	AE_{lip}	Fish > Crustacea	10.017	0.01	3.110	0.01
	AE_N	Fish > Squid	7.483	0.025	3.324	0.05
King Penguin	AE_e	Squid > Crustacea	8.934	0.025	2.9892	0.05
	AE_{eN}	Squid > Crustacea	7.134	0.05	2.590	0.05
	AE_{lip}	Squid > Crustacea	7.216	0.05	2.458	0.05
	AE_{Ca}	Squid > Crustacea	8.346	0.025	2.856	0.05
	AE_N	Fish > Crustacea	8.346	0.025	2.578	0.05

APPENDIX 4.3. Values of Kruskal-Wallace test statistics for intraspecific pairwise comparisons of predicted and observed metabolizable energy coefficients between different food types. > indicates which of the two food types resulted in a higher mean MEC value. In all cases, df = 2.

Species		Food types	H	P <	Y_m/S_m	P <
Cape Gannet	MEC_o	Fish > Crustacea	6.146	0.05	2.463	0.05
	MEC_p	Fish > Crustacea	13.346	0.005	3.642	0.01
Blue Petrel	MEC_o	Fish > Squid	6.877	0.05	2.596	0.05
	MEC_p	Fish > Squid	13.080	0.005	3.573	0.01
White-chinned Petrel	MEC_p	Fish > Squid	14.118	0.001	3.720	0.01
Sooty Albatross	MEC_p	Fish > Squid	12.756	0.005	3.501	0.01
Rockhopper Penguin	MEC_p	Squid > Crustacea	12.877	0.005	3.587	0.01
Gentoo Penguin	MEC_p	Fish > Crustacea	12.150	0.005	3.320	0.01
King penguin	MEC_p	Fish > Crustacea	10.154	0.01	2.830	0.05

CHAPTER 5

GASTROINTESTINAL TRANSIT AND LIPID ASSIMILATION EFFICIENCIES IN THREE SPECIES OF SUB-ANTARCTIC SEABIRD

With Allen R. Place, *J. Exp. Zool.* (in press)

SUMMARY

Using tritium-labeled glycerol triether ([^{3}H] GTE) as a non-absorbable lipid-phase marker and tritium-labeled polyethylene glycol 4000 ([^{3}H] PEG) as a non-absorbable aqueous-phase marker, we examined gastrointestinal transit of homogenized Antarctic Krill (*Euphausia superba*) meals fed to White-chinned Petrels (*Procellaria aequinoctialis*), Sooty Albatrosses (*Phoebetria fusca*) and Rockhopper Penguins (*Eudyptes chrysocome*). The aqueous-phase marker was excreted significantly more rapidly than was the lipid-phase marker by the two procellariiform species, whereas no differential transit rates for the two markers were observed in the penguins. Aqueous-phase marker recoveries after 48 hours from the three species were statistically indistinguishable (78.6% ± 3.7%, n = 5; 71.9% ± 11.3%, n = 7 and 77.0% ± 9.4%, n = 4 respectively). Lipid-phase marker recovery from the penguins after 48 hours was nearly complete (83.8% ± 19.3%, n = 5, and 92.7% ± 14.8%, n = 5 for two dietary lipid supplements, see below), whereas less than 50% of the original dose of lipid marker was recovered from the two procellariiform species. Substantial lipid-phase marker was recovered as stomach oils from the procellariiforms.

Assimilation efficiencies of [1-^{14}C] tripalmitin dissolved in wax ester and [1-^{14}C] cetyl oleate dissolved in triglyceride were compared for the same three seabirds by comparing ^{3}H/^{14}C ratios in the food and feces of birds simultaneously fed one of the above ^{14}C-labeled lipids, and the non-metabolizable marker [^{3}H] GTE. The petrel and the albatross showed high assimilation efficiencies (> 80%) of both ^{14}C-labeled neutral lipids. Rockhopper Penguins consistently excreted [^{3}H] GTE faster than did adult Sooty Albatrosses, and were significantly less efficient at assimilating both neutral lipids (62% and 45% respectively). Sooty Albatross fledglings excreted lipids significantly more slowly than did adults of this species, but lipid assimilation efficiencies did not differ with age. Gut measurements showed that the intestine of the Rockhopper Penguin was three and six times as long as those of the Sooty Albatross and White-chinned Petrel respectively.

INTRODUCTION

The role of lipids, especially wax esters, in marine food webs has interested marine ecologists for the past two decades (Lee *et al.*, 1971; Benson *et al.*, 1972; Benson and Lee, 1975, Sargent *et al.*, 1976; Bauermeister and Sargent, 1979). Because a high proportion of zooplankton productivity resides in wax esters (Benson and Lee, 1975), and wax esters are a major component of many seabird diets (Roby *et al.*, 1986), and of petrel and shearwater stomach oils (Cheah and Hansen, 1970a,b), knowledge of the proportion of ingested lipids that can be utilized by predators such as planktivorous seabirds would seem essential in studies of energy flow in marine ecosystems. Recent studies have revealed that petrels and alcids assimilate 80 to 95% of ingested wax esters (Obst, 1986; Place and Roby, 1986; Roby *et al*, 1986; Place and Butler, subm.). Although gastrointestinal passage rates of wax esters have been studied in southern giant petrel *Macronectes giganteus* and gentoo penguin *Pygoscelis papua* chicks (Roby *et al.*, 1989), there have been no published studies comparing assimilation efficiencies of different lipid classes in penguins or albatrosses. By virtue of their large populations and individual body sizes, members of both of these seabird groups are important secondary consumers in marine high latitude ecosystems (Croxall *et al.*, 1984). One goal of our study was thus to quantify lipid assimilation efficiencies in three species of Southern Ocean seabird, including an albatross and a penguin.

Our second goal was to investigate the relationship between gut morphology and gastrointestinal passage rates of lipid and aqueous dietary components in the same three species. Gut morphology varies considerably between seabirds (Mitchell, 1901; Imber, 1985), particularly between procellariiforms and penguins (Roby *et al.*, 1989). Although the taxonomic implications of variations in procellariiform gut morphology have been discussed by Imber (1985), the ecological significance of interspecific differences in gut morphology within this group has received little attention. Gastric morphology influences the rate of lipid passage

through procellariiform stomachs (Duke *et al.*, 1989; Place *et al.*, 1989; Roby *et al.*, 1989).

To monitor passage of digesta through the gastrointestinal tract we used two phase-specific nonabsorbable markers: polyethylene glycol (MW 4000) as an aqueous-phase marker (Wingate *et al.*, 1972), and glycerol triether as a lipid-phase marker (Morgan and Hofmann, 1970; Carlson and Bayley, 1972a, 1972b; Meyer *et al.*, 1986). Both markers are nontoxic and not degraded by digestive or bacterial enzymes, and do not influence the normal absorption of dietary aqueous nutrients or fat. To estimate lipid assimilation efficiencies we fed birds a C^{14}-labeled triglyceride or wax ester together with the ^{3}H-labeled nonabsorbable triether, and measured $^{3}H/^{14}C$ ratios in faecal and proventricular samples at intervals after feeding. This procedure circumvents problems arising from measurement of non-dietary (endogenous) lipids, as well as obviating the need for complete collection of feces.

The final goal of our study was to compare assimilation efficiencies and gastrointestinal transit times between procellariiform adults and chicks. Procellariiform chicks receive meals of higher energy-density than the food originally eaten by their parents (Ricklefs *et al.*, 1986). In addition, long intervals between visits to the nest by adult birds suggest that fledglings feed less frequently than do adult birds. These differences may influence digestive processes in birds of different ages. Gastric evacuation rates of lipids differ between adult and juvenile alcids (Roby *et al.*, 1986), but there have been no published studies investigating whether the same is true for procellariiforms. We therefore compare lipid assimilation efficiencies and gastro-intestinal passage rates in fledgling and adult Sooty Albatrosses.

MATERIALS AND METHODS

Study area and subjects

Feeding experiments were carried out at Marion Island (46°54'S; 37°45'E) from 31 March to 22 April 1988, during the annual relief voyage. Fifteen fledgling White-chinned Petrels (*Procellaria aequinoctialis*), six fledgling and twelve adult Sooty Albatrosses (*Phoebetria fusca*), and fifteen adult post-moult Rockhopper Penguins (*Eudyptes chrysocome*) were used. The birds were removed from their burrows or nest sites and held captive for 12-24 hours before the start of the experiments. Each bird was then fed a meal of Antarctic Krill *Euphausia superba* of a mass standardized to 6-8% of bird body mass. The average wet meal mass fed to individuals of each species was 95.1 ± 17.7g (n = 15) for White-chinned Petrels, 142.5 ± 6.5g for Sooty Albatross adults (n = 11), 144.7 ± 3.4g (n = 5) for Sooty Albatross fledglings, and 91.9 ± 9.7g (n = 15) for Rockhopper Penguins. These meal sizes were selected to fall within the range fed to chicks by adult White-chinned Petrels (Berruti *et al.*, 1985), Light-mantled Sooty Albatrosses *Phoebetria palpebrata* (Thomas, 1982) and Rockhopper Penguins (Brown and Klages, 1987). There are no published data on meal sizes for the Sooty Albatross.

Each species was divided into three groups of equal size, two of which were fed lipid solutions containing either [^{14}C] cetyl oleate (a wax ester) or [^{14}C] tripalmitin (a triglyceride) and the third a normal saline solution containing 50 mg/ml [^{3}H] polyethylene glycol (PEG). The two lipid solutions also contained [^{3}H] glycerol triether (GTE). Time and the number of available Sooty Albatross fledglings were limited, so in this case three birds were fed the [^{14}C] tripalmitin solution, and the other three [^{3}H] PEG. One Sooty Albatross adult fed [^{14}C] tripalmitin, and one Rockhopper Penguin fed [^{3}H] PEG regurgitated part of the meal shortly after feeding. These two individuals were released immediately, reducing the sample sizes for the two experimental groups to three and four respectively.

Birds were fed homogenized krill using 60 cm^3 plastic syringes attached to 15 cm lengths of plastic tubing with diameter 12 mm. The tubing was gently pushed approximately 10 cm into each bird's esophagus before emptying of the syringe contents. Each bird was fed half of the krill meal, followed by 4 ml of one of the three labeled substances, then the remaining krill. The labels were introduced directly into each bird's esophagus through a 15 cm length of tubing (diameter 2 mm) attached to a Gilson 5 ml micropipette. Immediately after feeding, the birds were confined in plastic barrels with mesh floors, raised over aluminium foil trays. The trays were changed 6, 12, 24 and 48 hours after feeding for the birds fed lipid markers, and 2, 4, 6, 8, 12, 24 and 48 hours after the feeding of normal saline. The collected feces were sealed in plastic bags, complete with foil trays, and frozen immediately at -20°C. Subsamples of krill were frozen at the same temparature for lipid analysis.

Proventriculus contents of each bird were sampled 24 hours after feeding, using suction through a tube (diameter 3 mm) inserted via the esophagus. At least 30 ml were withdrawn from each bird. To facilitate suction, it was necessary to dilute the proventriculus contents of the Sooty Albatrosses and Rockhopper Penguins with 30 ml of distilled water. Sooty Albatross fledglings were not sampled, as they were inclined to regurgitate when handled during the feeding trials. The proventricular contents of the White-chinned Petrel fledglings were withdrawn without dilution.

Lipid solutions

Refined olive oil, oleic acid and polyethylene glycol (PEG) (mol. wt 4000) were purchased from Sigma Chemicals (St Louis, MO, USA), and cetyl alcohol from Aldrich Chemicals, Milwuakee, WI, USA. Adult Antarctic Krill were captured by vertical net hauls off the Antarctic Peninsula in December 1987, and frozen for 4 months at -20°C before being thawed and homogenized. All other chemicals were reagent grade unless otherwise specified. All solvents were either pesticide or HPLC grade.

Radiolabels and fluors

We used tri-[1-^{14}C] palmitate (60 mCi/mmol) and [1-^{14}C] cetyl alcohol (24 mCi/mmol), obtained from Amersham (Arlington Heights, IL, USA). These chemicals had radio-purities > 98%, determined by thin-layer chromatography. [1,2-^{3}H] polyethylene glycol ([^{3}H] PEG) (1.4 mCi/mmol) was purchased from New England Nuclear (Boston, MA, USA). The use of [^{3}H] PEG as a non-absorbable aqueous marker has been validated for birds (Tur and Rial, 1985) and for humans (Wingate *et al.*, 1972).

Fluors were ACS II (Amersham, Arlington Heights, IL, USA), Biosafe II (Research Products International, Mount Prospect, IL, USA), and Readysafe (Beckman, Johannesburg, South Africa). Samples containing lipid-phase markers were counted on a Beckman LS 3801 scintillation counter, and aqueous-phase marker samples on a Packard Tri-Carb 460 scintillation counter. For both sets of samples, a correction ("quench") curve was derived using Compton edge ("H number") calibration (Beckman Instruments). Counting efficiency for ^{14}C in the samples varied from 88% to 74%, and for ^{3}H from 22% to 6.0%. Counting times (2 - 10 min) were chosen to ensure at least 95% counting accuracy. The coefficient of variation for replicate samples averaged 3.0% ± 1.3% for tritiated lipids, 1.9% ± 0.9% for carbon-14, and 3.9% ± 1.1% for tritiated PEG. All radioactivities are expressed in μCi (1.00 μCurie = 37.0 kilobequerals).

Tracer synthesis

Bachem Bioscience Inc. (Philadelphia, PA) synthesized the glycerol triether [1-(9 cis-octadecenyl) 2,3 didodecyl glycerol triether] as described by Morgan and Hofmann (1970). The tritiated glycerol triether ([^{3}H] GTE) was prepared by reduction with platinum as a catalyst (New England Nuclear, Boston, MA).

Purified [^{3}H] GTE (> 98% radiopurity) was obtained by chromatography on a silicic acid column eluted with hexane/diethyl ether 85:15 (v/v). Solvent was removed with nitrogen evaporation and the purified [^{3}H] GTE dissolved in absolute ethanol to a specific activity of 1 mCi/ml. Labeled wax ester ([1-^{14}C] cetyl oleate

and carrier cetyl oleate were synthesized as described by Place and Roby (1986), using p-toluene sulfonic acid catalyzed esterification. The labeled wax was purified on a 10 cm3 silicic acid column. The desired product was eluted with five to ten column volumes of 2% (v/v) diethyl ether in petroleum ether and solvent removed under nitrogen. The cetyl wax ester was dissolved in 250 µl toluene and stored under nitrogen at -20°C. Radiometric scanning of the TLC plate indicated that the radiopurity (2.2 mCi/mmol) of each lipid was 98.7%. Based on TLC/FID on Chromorods S-II (Ackman, 1981), using hexane:diethyl ether:formic acid (85:15:0.1), the chemical purity of the wax ester was greater than 98%.

Measurements of gastrointestinal tracts

After termination of the feeding trials, one adult Rockhopper Penguin, and one fledgling of each of the two procellariiform species, was killed under permit by intravenous injection of 5 - 10 ml of "Eutha-naze" (a stable solution containing 200 mg of sodium pentobarbitone per ml, Centaur Laboratories, Johannesburg, South Africa). All other adult birds used in the feeding experiments were released, and the fledglings returned to their nests. The three dead birds were frozen intact, and shipped to the University of Cape Town, where each bird was thawed and the abdominal cavity opened. The gastrointestinal tract was sketched in situ, then dissected out. Each part of the gastrointestinal tract was then weighed to the nearest 0.1 g, and measured to the nearest millimeter. In the penguin, the proventriculus was considered to be that portion of the gastric region covered with secretory cells. The procellariiform proventriculus and ventriculus (gizzard) are separated by a marked constriction (the isthmus), hence clearly distinguishable. The gizzard was considered as the part of the gastric region between the proventriculus and the pylorus. Prior to weighing, the mucus lining of the gizzard was removed. For both proventriculus and gizzard, maximal length and width (opened) were used to estimate area. Length of each intestinal part was measured with the segment fully extended but not stretched. Width of each intestinal part was

measured on opened segments at the proximal, mid- and distal sections. Area was estimated from the product of length and mean width.

Marker recovery and distribution

Each sample of excreta was weighed to the nearest 0.1 g prior to extraction. Excreta samples from [^{3}H] PEG-fed birds were washed from the foil and diluted to 100 ml total volume with double-distilled water. One-milliliter aliquots were removed immediately for scintillation counting. Accumulated excreta from each foil tray were extracted with chloroform:methanol (2:1), filtered on glass fiber filters to obtain a single phase extract, then washed with 0.25 volumes of 0.9% KCl (w/v) (Bligh and Dyer, 1959). The lipid-containing lower phase was separated and its volume measured. Aliquots of the lower chloroform phase (1 ml) were placed in scintillation vials and the solvent removed under nitrogen evaporation. Fluor was added and the contents of the vials counted as described above.

Assimilation of the [^{14}C]-labeled lipid by the birds was calculated from the ratio of [^{3}H]-marker to [^{14}C]-marker in the fecal collections, using the formula:

(1) % assimilation efficiency = 1 - [(^{3}H/^{14}C in test meal) per (^{3}H/^{14}C in fecal collection)] X 100

The distribution of label among the various fecal and stomach oil lipid classes was determined by TLC of the lipid extracts. Aliquots containing equivalent counts were spotted on the pre-absorbent area of channelled silica G plates (Uniplates, Analtech). After development with hexane:diethyl ether:acetic acid (80:20:1), the plate was scanned with a Vanguard radiometric scanner. This solvent system resolves wax esters, triacylglycerols, fatty acids, fatty alcohols, 1,3-diacylglycerols, 1,2-diacylglycerols, monoacylglycerols and complex lipids in order of decreasing refractive index (Rf) (Place and Roby, 1986). To separate cholesterol esters from wax esters, double development with hexane:diethyl ether (98:2) was necessary (Christie, 1982). The carrier gas used in the radiometric scanning was Q-gas (1.3% n-butane with the remainder helium) at a flow rate 0.5 - 1.0 liters/min. The spatial resolution for each scan was set at 2 mm. The distribution of each label among the

lipid classes was estimated by integration of the counts under each peak after subtraction for background. The overall counting efficiency for ^{14}C averaged 10.5% whereas that for ^{3}H averaged 1.2% across the plate. Labeled cetyl oleate, cholesterol oleate, triolein, oleic acid, and cetyl alcohol were used as standards to determine the Rf of these major lipid classes.

Gastrointestinal transit

Food retention time was estimated using the exponential function:

$$nt = b_0 - e^{(-b_1 X (time - b_2))} \quad (2)$$

where n_t is the proportion of marker excreted from initial administration to time t (Hove, 1984). The parameter b_0 is the asymptotic recovery of marker, b_1 is the rate of excretion (h^{-1}), and b_2 is the delay in hours before the marker is recovered. The total mean retention time is equal to the sum of b_2 and the reciprocal of b_1. Estimates and 95% confidence intervals for the three parameters were obtained by weighted nonlinear least squares regression weighted by the variance of replicates (Johnson *et al.*, 1981).

Statistics

Results are expressed as means with one standard deviation, and "n" represents the sample size. Non-parametric statistical tests were used throughout. Two-tailed Wilcoxon U-tests were used for pairwise comparisons. Differences were considered significant when $P \leq 0.05$, except for comparisons involving ratios of the disintegration per minute (DPM) of two markers, for which $P \leq 0.001$ was chosen. In no cases were fewer than 1000 DPM per sample for either isotope observed. Curves were calculated by weighted nonlinear least squares iterative procedure (modified Gauss-Newton method, Johnson *et al.*, 1981). Parameter estimates for equation (2) are given with their 95% confidence limits in parentheses.

RESULTS

Meal water and lipid content

The wet mass to dry mass ratio for the krill was 4.25 ± 0.04 (n = 6), and the lipid content (% wet weight) was 4.7 ± 0.5% (n = 6). The two major lipid classes (by percentage weight of total lipids) were triacylglycerol (30.3 ± 4.6%, n = 6) and phospholipid (40.7 ± 2.6%, n = 6). These figures are consistent with other analyses of krill composition (Clarke, 1980; Fricke *et al.*, 1984).

Aqueous and lipid marker recovery

The extent of dilution of the [^{3}H] PEG by gastric juices in the proventriculi of the seabirds was unknown. Consequently, no attempt was made to estimate absolute amounts of the aqueous marker remaining in the proventriculus 24 hours after feeding. For all three seabird species, similar percentages (70 - 78%) of the original [^{3}H] PEG doses were recovered after 48 hours.

(a) Comparison between adults: Rockhopper Penguins and Sooty Albatrosses

The function best describing the rate of gastrointestinal emptying of [^{3}H] PEG, for these two seabird species was fitted using equation (2) (Fig. 5.1a). The rates of passage (b_1) for aqueous components are 0.39 (0.31 - 0.47) h^{-1} and 0.16 (0.13 - 0.19) h^{-1} for Rockhopper Penguin and Sooty Albatross adults respectively. The times for first appearance (b_2) of the aqueous marker are 1.2 (1.1 - 1.3) hours and -0.6 (-1.1 - - 0.02) hours respectively. We interpret negative values of b_2 to indicate a zero delay for first appearance of the marker. The total mean retention times for aqueous components are thus 3.8 and 6.3 hours for the penguin and the albatross respectively. There were no significant differences between cumulative percentages of [^{3}H] PEG excreted by the two species at any of the time intervals.

Amongst birds fed the [^{14}C]-labeled triglyeride solution, Rockhopper Penguins excreted a significantly higher proportion (83.8 ± 19.3%) of the original [^{3}H] GTE dose than did Sooty Albatrosses (36.3 ± 15.9%, $U_{4,5} = 20$, $P \leq 0.02$). The same is

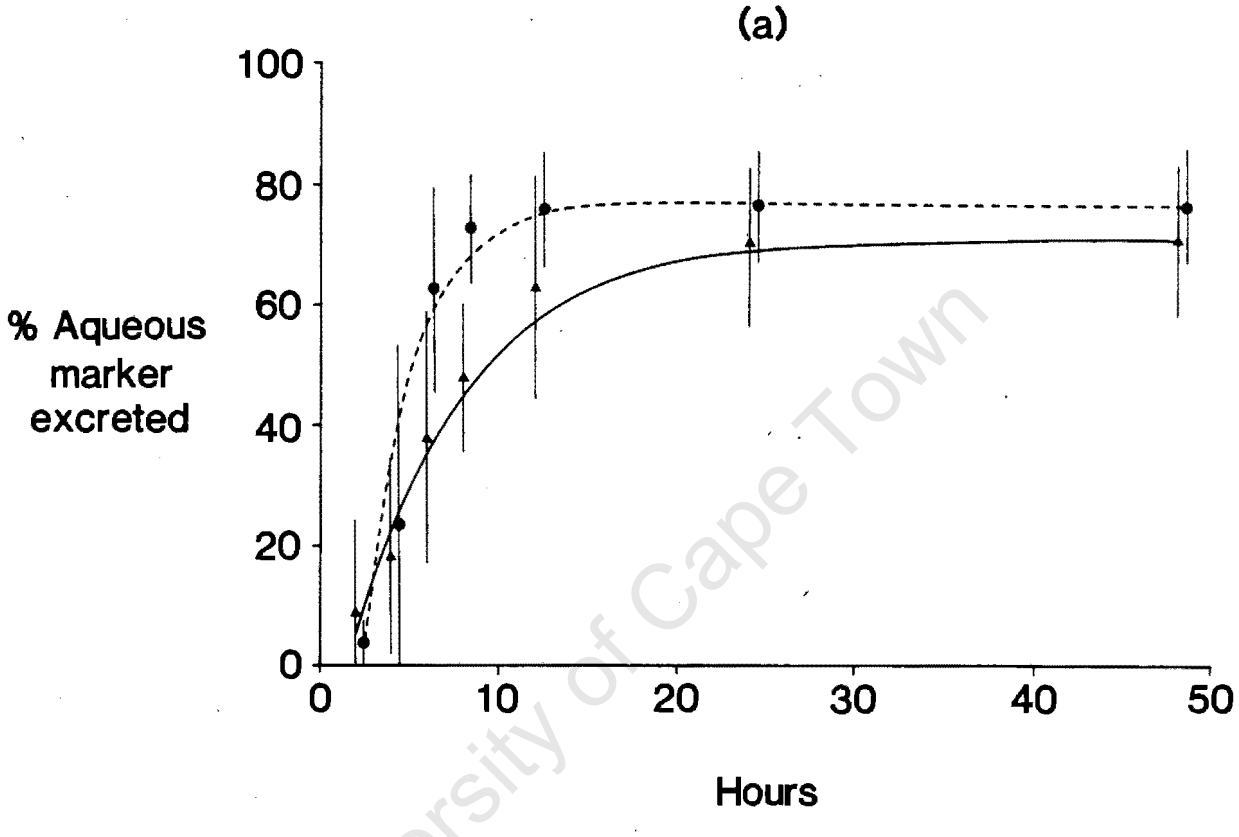

Figure 5.1 (a). Cumulative excretion of the aqueous-phase marker [1-^{3}H] PEG by adult Sooty Albatrosses (*Phoebetria fusca*) (▲—▲) and Rockhopper Penguins (*Eudyptes chrysocome*) (●--●). The exponential function describing the fitted curves is equation (2):

$$n_t = b_0 - e^{(-b_1 \times (\text{time} - b_2))}$$

where n_t equals the proportion of marker recovered from initial administration to time t, b_0 is the asymptote, b_1 the rate of marker excretion per hour, and b_2 the delay in hours before first recovery of the marker. Values of these parameters are given in the text. The bars represent one standard deviation.

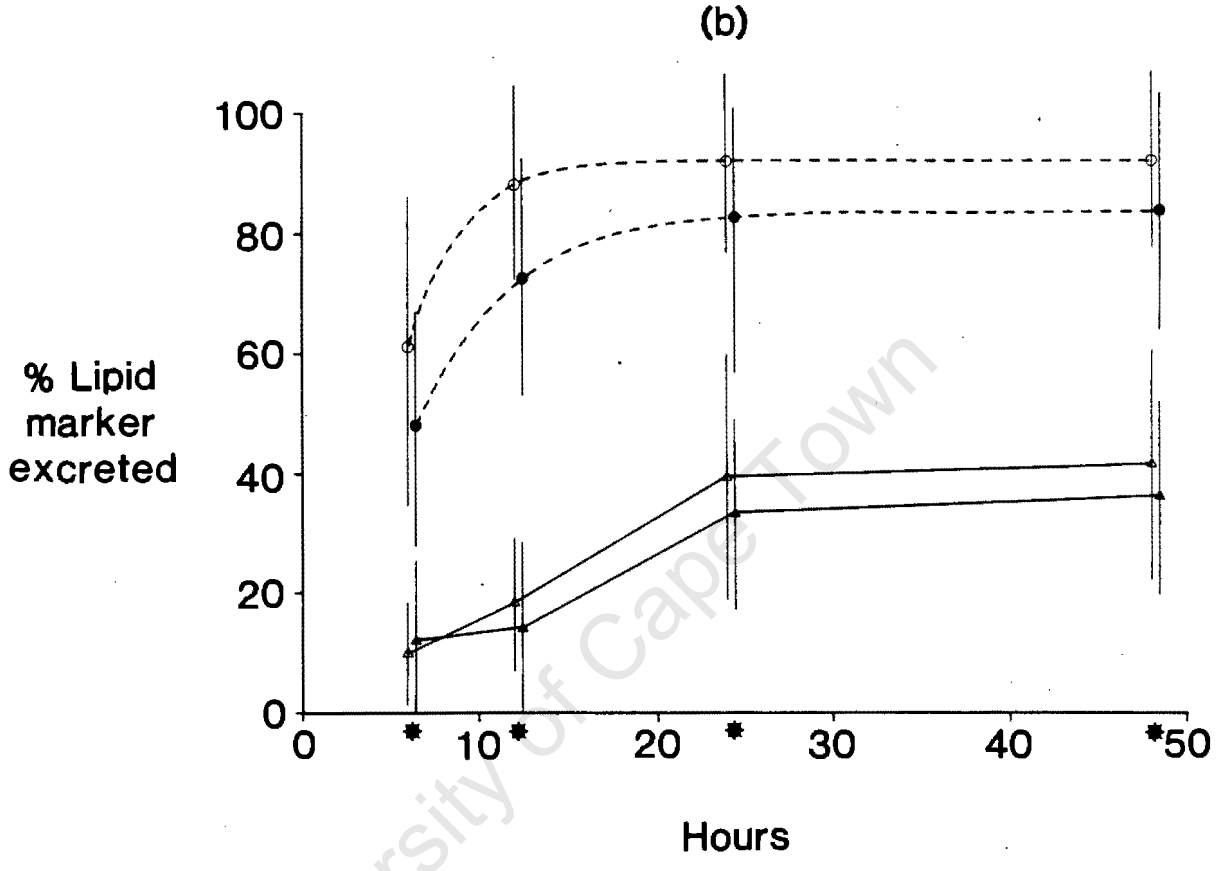

Figure 5.1 (b). Cumulative excretion of the lipid-phase marker [^{3}H] GTE by adult Sooty Albatrosses and Rockhopper Penguins. Symbols and equation as as for Fig. 5.1 (a), with solid symbols denoting birds fed the wax ester carrier solution (^{14}C triglyceride), and hollow symbols birds fed the triglyceride carrier solution (^{14}C wax ester). Asterisks on the x-axis denote significant differences in cumulative marker or lipid recovery between the two species at the specified time intervals (see results section (a)).

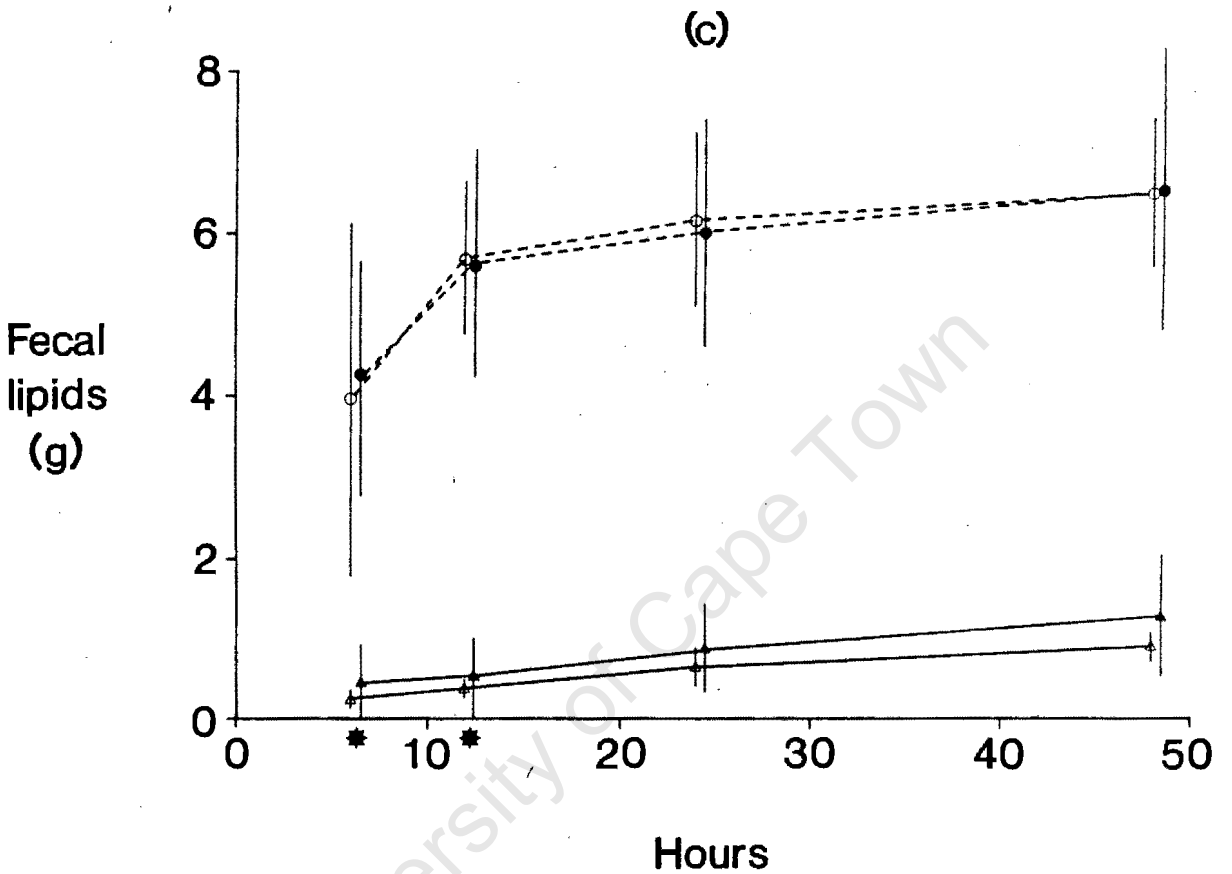

Figure 5.1 (c). Cumulative excretion of lipids (g) by adult Sooty Albatrosses and Rockhopper Penguins. Symbols as as for Figs 5.1 (a) and (b).

true of birds fed the [^{14}C]-labeled wax ester (total marker recoveries of 92.7 ± 14.7% and 41.5 ± 19.1% respectively for the two species; $U_{3,5} = 15$, $P \leq 0.05$). These differences in [^{3}H] GTE recovery between the two species were consistent and significant at all time intervals, with U-values equal to those given above.

Curves representing gastrointestinal emptying rates of [^{3}H] GTE in the Rockhopper Penguins were fitted using equation (2) (Fig. 5.1b). The estimated rates of passage (b_1) for lipid components for birds fed labeled wax ester and triglyceride were 0.20 (0.15 - 0.27) h^{-1} and 0.34 (0.31 - 0.38) h^{-1} respectively. The times for first appearance of the marker (b_2) are 0.99 (-0.29 - 2.3) hours and 2.7 (2.4 - 3.0) hours respectively for the two treatments. The estimated total mean retention times for lipid components in penguins fed wax ester and triglyceride are 6.0 and 5.6 hours respectively. No attempt was made to fit curves to the lipid marker excretion data from the two procellariiform species, because gastric retention of the marker in stomach oils would confound interpretation of the fitted curves.

Among birds fed the [^{14}C]-labeled triglyceride solution, Rockhopper Penguins excreted significantly more fecal lipid after 6 and 12 hours than did Sooty Albatross adults (Fig. 5.1c, $U_{4,5} = 20$, $P \leq 0.02$ in both cases). This is also true for birds fed the [^{14}C]-labeled wax ester solution ($U_{3,5} = 15$, $P \leq 0.05$ for the same two time intervals).

For both lipid solutions, Sooty Albatross adults assimilated the two [^{14}C]-labeled lipids more efficiently than did the penguins (Fig. 5.2; $U_{4,5} = 20$, $P \leq 0.02$ between birds fed [^{14}C] tripalmitin; and $U_{3,5} = 15$, $P \leq 0.05$ between those fed [^{14}C] cetyl oleate).

Across all three species, high assimilation efficiencies were associated with low excretion rates of both neutral lipids, and vice versa (Fig. 5.2).

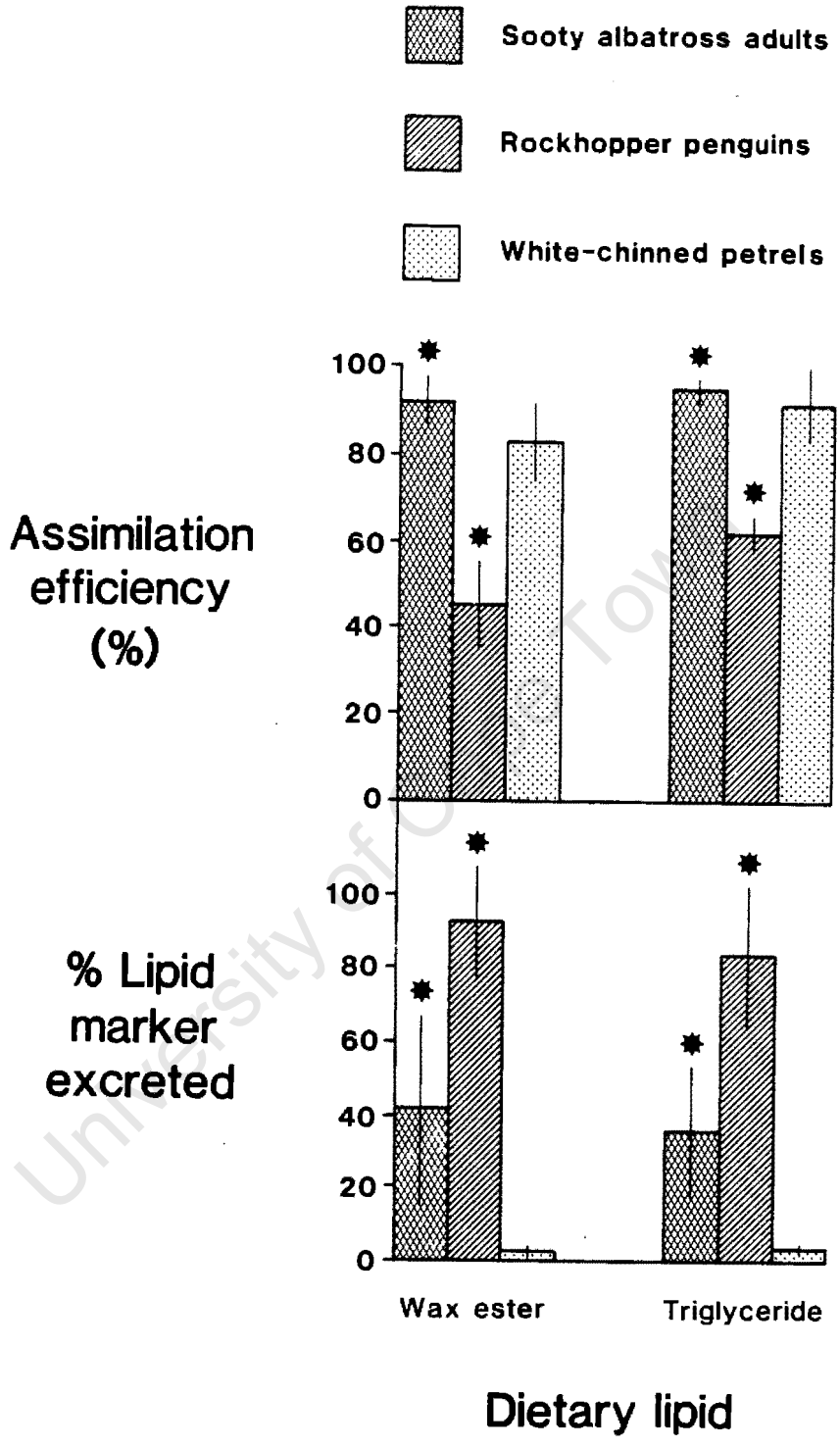

Figure 5.2. Assimilation efficiencies of neutral ^{14}C-labeled lipids (top) and excretion of lipid-phase marker ([^{3}H] GTE after 48 hours (bottom) by White-chinned Petrel fledglings, (*Procellaria aequinoctialis*), and Sooty Albatross (*Phoebetria fusca*) and Rockhopper Penguin adults (*Eudyptes chrysocome*). The bars represent one standard deviation. Asterisks denote significant differences in lipid assimilation efficiency and cumulative marker recovery between adult Sooty Albatrosses and Rockhopper Penguins (see results section (a)).

(b) Comparison between fledglings: White-chinned Petrels and Sooty Albatrosses fed [^{14}C] tripalmitin

The rates of passage (b_1) of aqueous components for White-chinned Petrel and Sooty Albatross fledglings are 0.23 (0.21 - 0.24) h^{-1} and 0.11 (0.09 - 0.14) h^{-1} respectively. Times for first appearance of the aqueous marker (b_2) for the two species are 0.9 (0.9 - 1.0) hours and 3.2 (2.8 - 3.6) hours respectively (Fig. 5.3a). Mean retention times of the aqueous marker are therefore 5.3 and 12.3 hours for White-chinned Petrel and Sooty Albatross fledglings respectively. Totals of 78.6 ± 3.7% and 73.6 ± 12.1% of the original [^3H] PEG doses were recovered after 48 hours from White-chinned Petrels and Sooty Albatrosses respectively. Significantly higher cumulative percentages of the aqueous marker were excreted by White-chinned Petrels at the 6, 8 and 12 hour intervals (Fig. 5.3a, $U_{3,5} = 15$, $P \leq 0.05$ in all cases).

Forty-eight hours after feeding, 3.7 ± 1.6% and 1.7 ± 1.5% of the original [^3H] GTE doses were excreted by White-chinned Petrel and Sooty Albatross fledglings respectively (Fig. 5.3b). Although total percentage lipid marker recovery was statistically indistinguishable between the two species, Sooty Albatross fledglings had excreted significantly less [^3H] GTE (0.20 ± 0.01%) after 12 hours than had the White-chinned Petrels (1.54 ± 1.10%; $U_{3,5} = 15$, $P \leq 0.05$). There were no significant differences between [^{14}C] tripalmitin assimilation efficiencies, and between the total amounts of fecal lipids excreted by fledglings of these two species (Fig 3c).

(c) Comparison between adult and fledgling Sooty Albatrosses

For both adult and fledgling Sooty Albatrosses, similar proportions of the aqueous marker were recovered in total (70.7 ± 12.4% and 73.6 ± 12.1% respectively). Cumulative percentages of marker excreted were significantly higher in adults for the six hour fecal collection (Fig. 5.4a, 38.1 ± 20.7% compared to 3.0 ± 2.8% in the fledglings; $U_{3,4} = 12$, $P \leq 0.05$). Curves describing the rates of gastrointestinal emptying of the aqueous marker (Fig. 5.4a) were fitted using

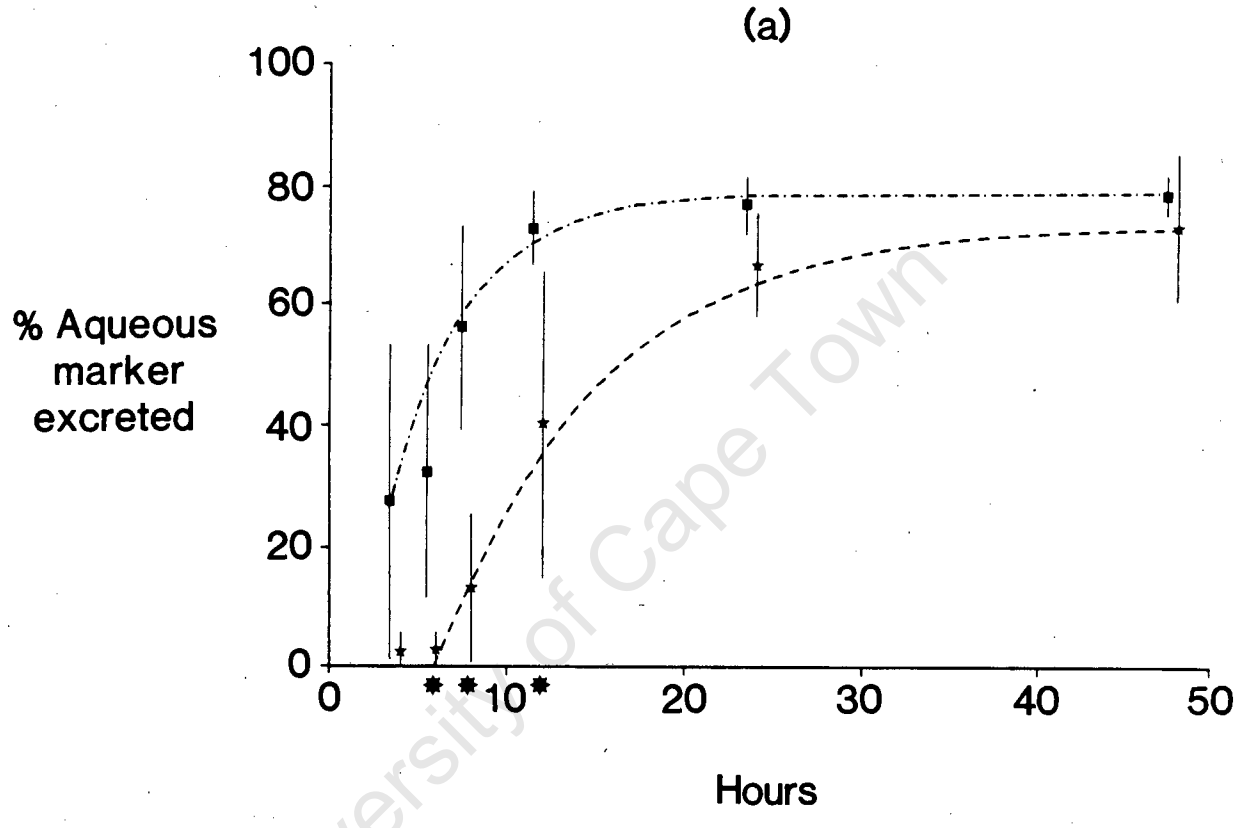

Figure 5.3 (a). Cumulative excretion of the aqueous-phase marker [^{3}H] PEG by fledgling White-chinned Petrels *Procellaria aequinoctialis* (■--■) and Sooty Albatrosses *Phoebetria fusca* (★--★). The equation for the function describing the curves is given in the caption for Fig. 5.1. The bars represent one standard deviation. Asterisks on the x-axis denote significant differences in cumulative marker or lipid recovery between the two species at the specified time intervals (see results section (b)).

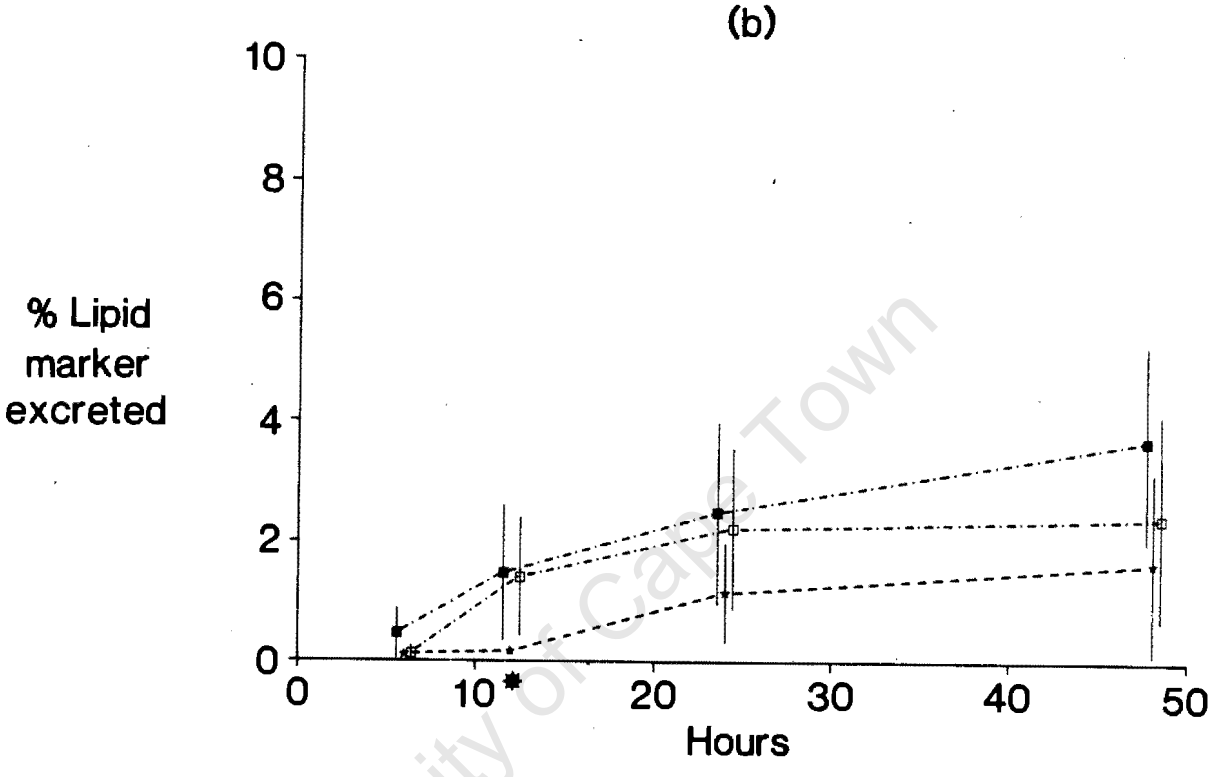

Figure 5.3 (b). Cumulative excretion of lipid-phase marker [^{3}H] GTE by fledgling White-chinned Petrels *Procellaria aequinoctialis* and Sooty Albatrosses *Phoebetria fusca*. Symbols as for Fig. 5.3 (a), with solid symbols denoting birds fed the wax ester carrier solution (^{14}C triglyceride), and hollow symbols birds fed the triglyceride carrier solution (^{14}C wax ester).

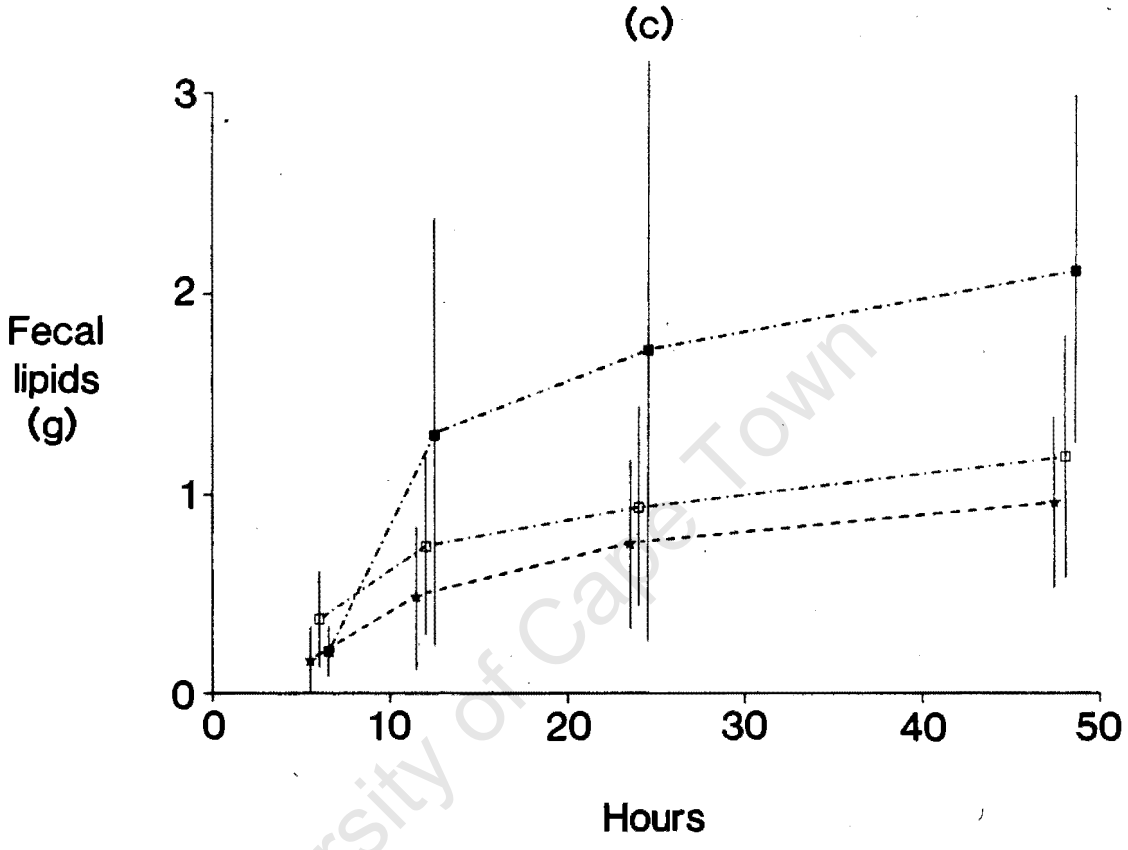

Figure 5.3 (c). Cumulative excretion of lipids (g) by fledgling White-chinned Petrels *Procellaria aequinoctialis* and Sooty Albatrosses *Phoebetria fusca*. Symbols as for Fig. 5.3 (a) and (b).

equation (2). Values of b_1 and b_2 for adults and fledglings of this species are given in the above two sections.

Pairwise comparisons between Sooty Albatross adults and fledglings reveal that the fledglings excreted significantly less [^{3}H] GTE at each time interval than did the adults (Fig. 5.4b, in all cases, $U_{3,4} = 12$, $P \leq 0.05$). Moreover, the total quantity of fecal lipids recovered from fledglings was significantly less than that recovered from adults ($U_{3,4} = 9$, $P \leq 0.05$) (Fig. 5.4c).

Despite the slower passage rates of the non-absorbable lipid marker, assimilation efficiencies of the 14-labeled triglyceride did not differ significantly between adults and fledglings (92.2 ± 5.1% and 96.3 ± 3.4% respectively).

Comparison between aqueous and lipid marker excretion by all species

After both 24 and 48 hours, significantly more of the aqueous than of the non-absorbable lipid marker had been excreted by White-chinned Petrels ($U_{5,5} = 50$, $P \leq 0.001$), Sooty Albatross adults ($U_{4,7} = 28$, $P \leq 0.005$), and Sooty Albatross fledglings ($U_{3,3} = 9$, $P \leq 0.05$). There were, however, no significant differences between the cumulative percentages of these two markers excreted by Rockhopper Penguins ($U_{5,10} = 25$, $P > 0.05$). For the above comparison, cumulative percentages of [^{3}H] GTE recovered from the birds fed ^{14}C-labeled wax ester, and from those fed the ^{14}C-labeled triglyceride, were statistically indistinguishable and therefore pooled. The lack of significant differences between the quantities of lipid marker excreted by wax ester- and by triglyceride-fed birds of all species suggests that the nature of the ^{14}C-labeled lipid did not influence the passage rate of [^{3}H] GTE.

Assimilation efficiencies of wax esters in comparison with triglycerides

Assimilation efficiencies of the ^{14}C-labeled wax ester and triglyceride for the three bird species (Fig. 5.2) were estimated using ^{3}H/^{14}C ratios in the food and feces. White-chinned Petrels assimilated the fatty acid moiety of cetyl oleate significantly less efficiently than they did the homologous fatty acid moiety of

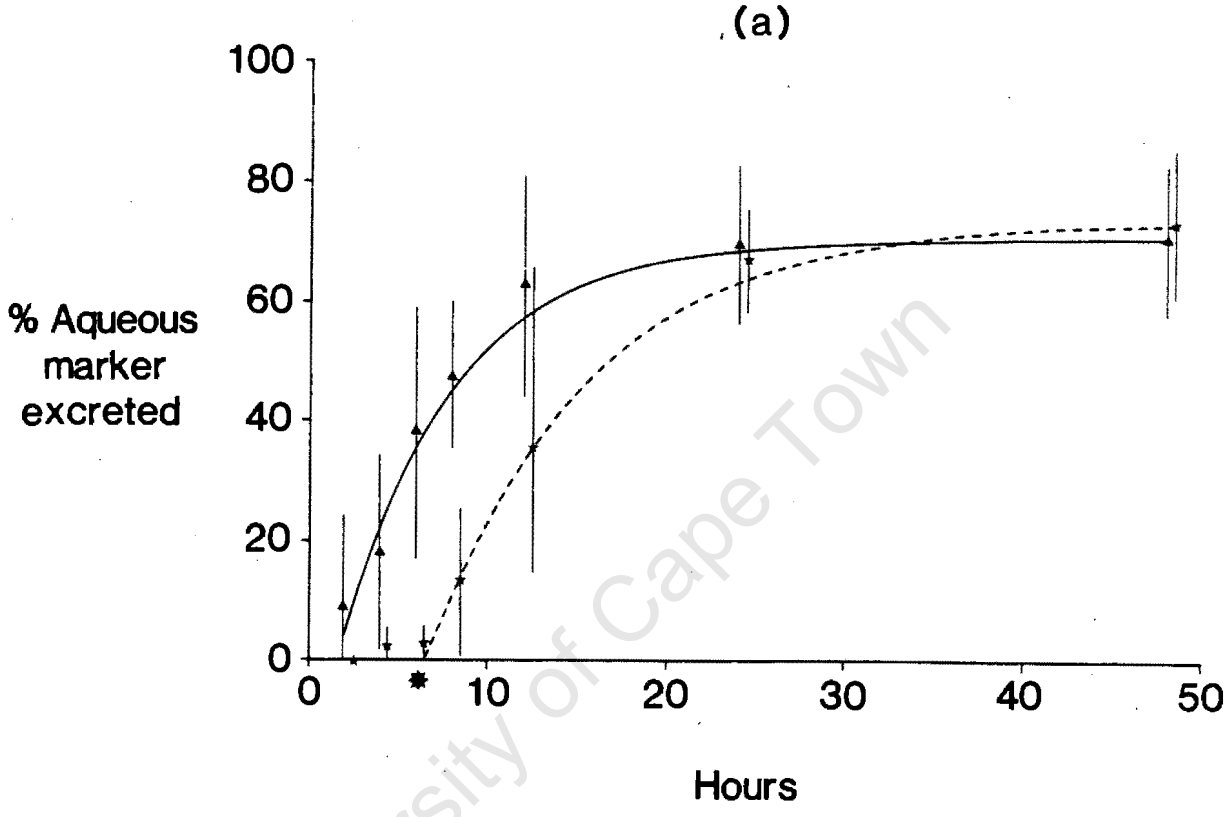

Figure 5.4 (a). Cumulative excretion of the aqueous-phase marker [^{3}H] PEG by adult (▲—▲) and fledgling (★--★) Sooty Albatrosses (*Phoebetria fusca*) fed the wax ester carrier solution (^{14}C triglyceride). The equation for the function describing the curves is given in the caption for Fig. 5.1 (a). The bars represent one standard deviation. Asterisks on the x-axis denote significant differences in cumulative marker or lipid recovery between adults and fledglings at the specified time intervals (see results section (c)).

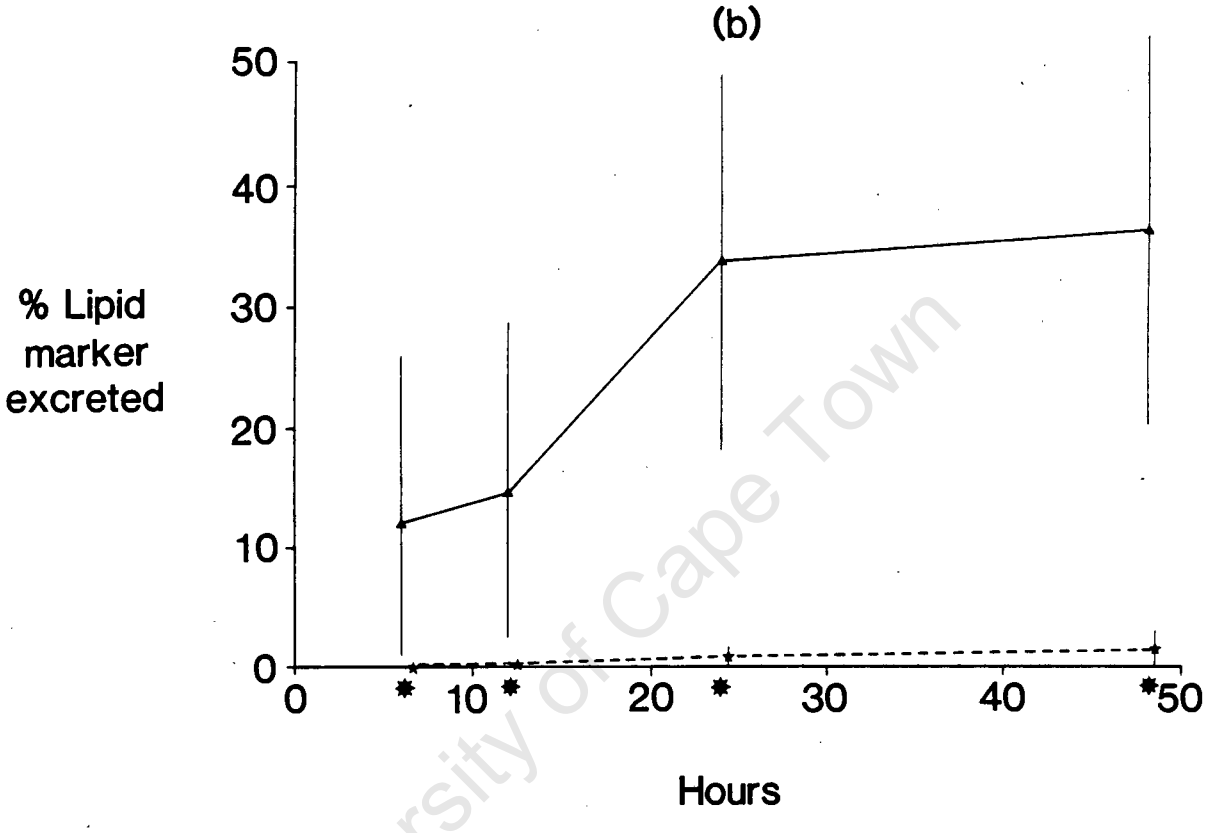

Figure 5.4 (b). Cumulative excretion of the lipid-phase marker [^{3}H] GTE by adult and fledgling Sooty Albatrosses (*Phoebetria fusca*) fed the wax ester carrier solution (^{14}C triglyceride). Symbols as for Fig. 5.4 (a).

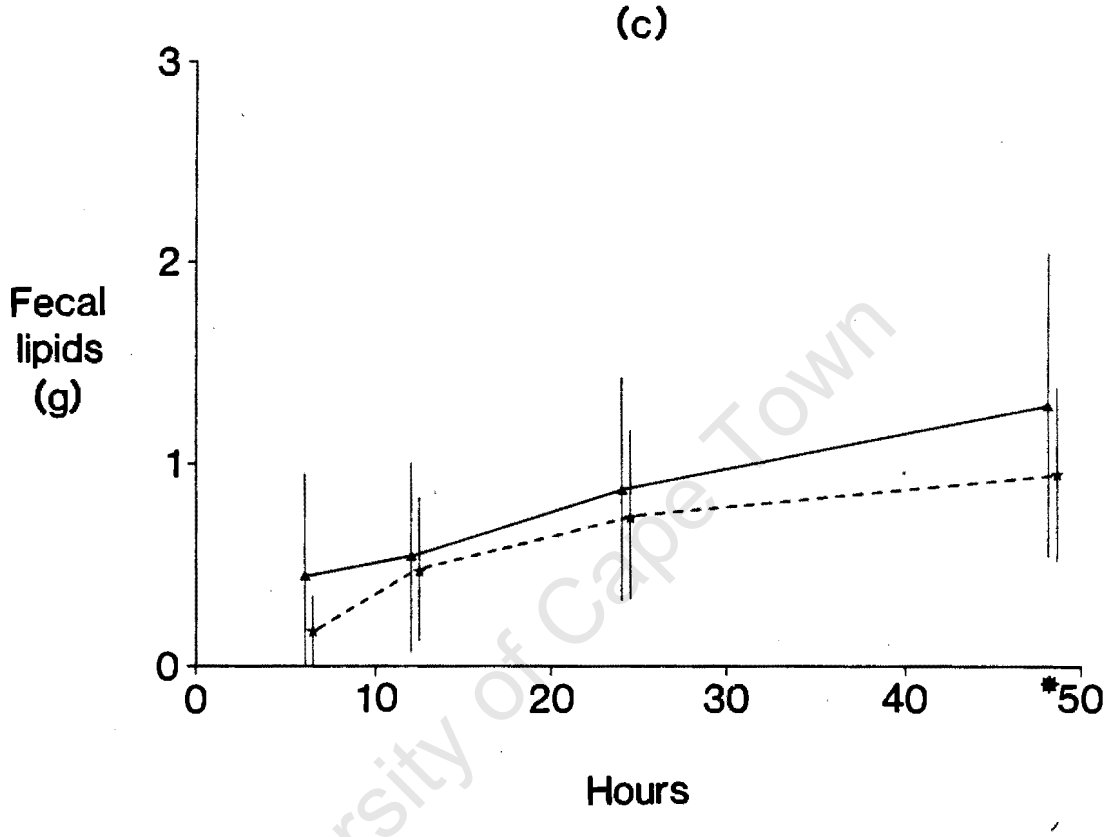

Figure 5.4 (c). Cumulative excretion of lipids by adult and fledgling Sooty Albatrosses (*Phoebetria fusca*) fed the wax ester carrier solution (^{14}C triglyceride). Symbols as for Figs 5.4 (a) and (b).

tripalmitin ($U_{5,5} = 21$, $P \leq 0.05$), and the same is true of Rockhopper Penguins ($U_{5,5} = 25$, $P \leq 0.005$). Assimilation efficiencies of other moieties within each species were statistically indistinguishable between birds fed the triglyceride and the wax ester carrier solutions.

Assimilation efficiencies estimated from lipid balance (i.e. [lipid fed - lipid excreted]/lipid fed) are similar to those estimated by the marker ratio technique. Sooty Albatross adults retained 91.3% of dietary lipid when fed meals containing wax ester carrier, and 87.5% when fed meals containing triglyceride carrier. Sooty Albatross fledglings retained 90.9% of dietary lipid when fed triglyceride-supplemented meals. Similarly, White-chinned Petrels retained 84.4% and 73.9% of dietary lipids respectively in the two treatments. Rockhopper Penguins, however, retained only 20.5% and 17.4% of dietary lipids respectively. In all three species, more than 80% of the defecated carrier lipid had been hydrolyzed.

Gastric lipolysis of stomach oils

Percentages of the original doses of ^{14}C label remaining in the proventriculus 24 hours after feeding were $0.14 \pm 0.16\%$ and $0.60 \times 10^{-4}\%$ respectively in White-chinned Petrel fledglings and Sooty Albatross adults fed ^{14}C-labeled wax ester; and $0.07 \pm 0.05\%$ and $2.5 \times 10^{-4}\%$ in the same two species fed ^{14}C-labeled triglyceride. Since the tritium label resided in a non-metabolizable, non-absorbable lipid marker, and the carbon-14 in a digestible triglyceride or wax ester, we expected that hydrolysis of the latter would lead to an increased $[^3H]/[^{14}C]$ ratio as ^{14}C-labeled triglycerides or wax esters emptied from the proventriculus. The initial $[^3H]/[^{14}C]$ ratio of wax ester fed to the birds was 3.08 ± 0.19 ($n = 10$), and that for the triglyceride-fed birds was 3.15 ± 0.19 ($n = 10$). Stomach lipids were recovered in quantity only from White-chinned Petrels. After 24 hours the $[^3H]/[^{14}C]$ ratios in stomach oils from this species were 3.40 ± 0.27 ($n = 5$) and 3.60 ± 0.96 ($n = 5$) respectively for the two treatments. For Sooty Albatrosses the ratios of $([^3H]/[^{14}C]$ in stomach oils were 3.64 ± 1.36 ($n = 5$) and 3.49 ± 2.15 ($n = 5$), respectively. From these figures we estimated an average gastric lipolysis of $11.8\% \pm 2.8\%$ in the two

procellariiform species. Based on dilution of the [^{3}H] GTE specific activity (μCi/ml of neutral lipid), we estimate that the White-chinned Petrels retained a mean of 14.4 ml (triglyceride-fed) and 27.5 ml (wax ester-fed) of stomach oil after 24 hours. These estimates include the quantity of neutral lipid fed plus lipid present at the time of feeding.

No lipids were recovered from the proventriculi of the Rockhopper Penguins 24 hours after ingestion of the labeled lipid solution, and scintillation counts from these samples were barely above background level.

Gastrointestinal morphology

Figs. 5a, b and c show *in situ* ventral views of the guts of the White-chinned Petrel, Sooty Albatross and Rockhopper Penguin, respectively. Looping of the ventriculus and duodenum are not as pronounced in the Sooty Albatross as in the White-chinned Petrel. The isthmus between the proventriculus and ventriculus is situated on the posterior surface of the proventriculus of the albatross, but on the ventral surface in the petrel, and is narrower in the petrel than in the albatross. The pyloric sphincter in the albatross is on the anterior surface of the ventriculus, and in the petrel it is on the posterior ventral surface. The Rockhopper Penguin has a poorly defined constriction between the two gastric compartments, and the pylorus is anterior and slightly dorsal to the ventriculus. In appearance, the stomach of the Sooty Albatross is intermediate between those of the White-chinned Petrel and the Rockhopper Penguin.

The gastrointestinal tract of the Rockhopper Penguin was both longer and heavier than those of the two procellariiforms (Table 5.1). The most marked differences in length, weight and surface area were between the small intestines: in the penguin this organ was three times as long as in the albatross, and almost six times as long as in the petrel. The White-chinned Petrel proventriculus was thin-walled and distensible, with the greatest surface area among the three species.

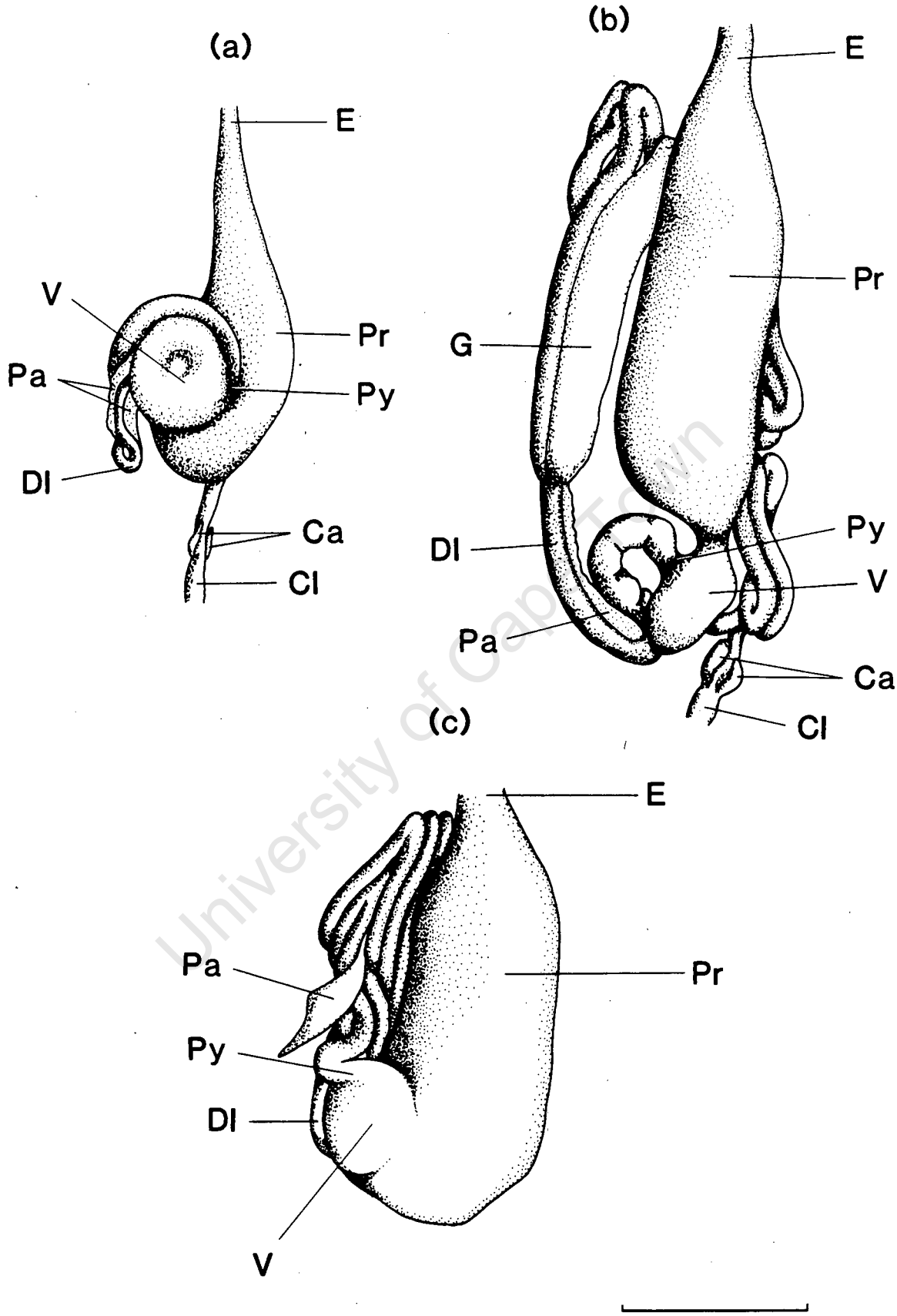

Figure 5.5. Ventral views of the gastro-intestinal tracts of (a) the White-chinned Petrel (*Procellaria aequinoctialis*), (b) the Sooty Albatross (*Phoebetria fusca*) and (c) the Rockhopper Penguin (*Eudyptes chrysocome*). The sterna, abdominal walls and livers have been removed. The scale bar represents 5 cm. Ca, ceca; Cl, colon; Dl, duodenal loop; E, esophagus; Pa, pancreas; Pr, proventriculus; Py, pylorus; V, ventriculus (gizzard).

Table 5.1. Measurements of the gastro-intestinal tracts of the White-chinned Petrel, Sooty Albatross and Rockhopper Penguin. Figures in parentheses after species names = individual body mass (wet, g).

Organ	White-chinned petrel (1 350)	Sooty albatross (2 150)	Rockhopper penguin (2 000)
WET MASS (g)			
Proventriculus	8.17	16.5	15.3
Ventriculus	1.8	2.3	14.4
Small intestine	3.4	17.0	45.6
Colon	0.3	0.8	1.3
Gall bladder	n.d.	7.1	2.7
Total	13.7	43.7	79.3
LENGTH (cm)			
Proventriculus	9.8	9.2	5.5
Ventriculus	2.6	2.6	7.5
Small intestine	78.0	145.0	465.8
Colon	6.5	6.9	9.6
Ceca	0.6 (l)	1.1 (l)	1.6 (l)
	0.6 (r)	0.6 (r)	1.3 (r)
Gall bladder	n.d	6.8	7.8
Total	96.9	163.7	488.4
PLANAR SURFACE AREA (length x width, cm^2)			
Proventriculus	69.3	47.5	55.0
Ventriculus	12.7	8.8	71.3
Small intestine	70.2	246.5	372.6
Colon	4.6	8.3	10.6
Ceca	0.2 (l)	0.3 (l)	1.9 (l)
	0.2 (r)	0.2 (r)	0.5 (r)
Gall bladder	n.d.	4.8 cm^3	4.7 cm^3
Total	157.2	311.6	511.9

*: Totals for length exclude the ceca and gall bladder, and totals for planar surface area exclude the gall bladder.
n.d.: no data

DISCUSSION

Differential passage of lipid and aqueous digesta: gastric morphology, lipolysis and stomach oil formation

The origin and functions of procellariiform stomach oils in have been well documented (Imber, 1976; Warham, 1977; Jacob, 1982 and Place *et al*, 1989), and their formation is known to be a function of lipid digestibility (Cheah and Hansen, 1970a). As the site of chemical digestion, the procellariiform proventriculus is functionally unique: in other birds, including penguins, mechanically-aided chemical breakdown of ingesta occurs in the ventriculus or gizzard (Duke, 1986). Chemical digestion of different dietary components proceeds at different rates, and lipid accumulation in petrel and shearwater stomachs is the result of simultaneously slow lipolysis and rapid proteolysis in an acid medium (Cheah and Hansen, 1970a). Previous work on wax ester assimilation in Leach's Storm-Petrels *Oceanodroma leucorhoa* (Place and Roby, 1986; Place *et al*., 1989) indicated that little ($\leq 5\%$) of wax ester lipolysis occurred in the proventriculus, even after 36 hours. We estimate similar low values ($\leq 12\%$) of gastric lipolysis in White-chinned Petrels and Sooty Albatrosses. Tritium to carbon-fourteen ratios in the stomachs of the two procellariiforms 24 hours after feeding were only slightly lower than the original ratios of the solutions fed to the birds, indicating slow rates of gastric lipolysis of the ^{14}C-labeled neutral lipids.

Procellariiform stomachs are morphologically distinctive. The gross anatomy of the White-chinned Petrel gastrointestinal tract reported here resembles those described for the congeneric Parkinson's Petrel *Procellaria parkinsoni* (McLelland, 1979) and for the southern giant petrel (Roby *et al*., 1989). Several studies have indicated that gastric morphology is instrumental in stomach oil formation: both the structure and limited motility of the stomach contribute to layering of lipid and aqueous digesta in Leach's storm-petrels (Duke *et al*., 1989; Place *et al*., 1989) and subsequent accumulation of stomach oil. Differential passage rates of lipid and aqueous digesta in Southern Giant Petrel and Gentoo Penguin chicks are related to

stomach structure (Roby *et al.*, 1989). In the petrel, ventral positioning of the pyloric sphincter, and looping of the ventriculus and duodenum, resulted in drainage of the aqueous substances from the stomach, and long retention times of relatively less dense lipid digesta. The penguin duodenum was not as sharply looped, and the slightly dorsal position of the pyloric sphincter prevented the separation of lipid and aqueous digesta that occurred in the petrel. We have described similar differences between the gastric morphology of the Sooty Albatross and Rockhopper Penguin which probably account for the differences in gut passage times of both ^{14}C-labeled neutral lipids and fecal lipids between adults of these two species.

The positioning of the pylorus is instrumental in stomach oil accumulation (Duke *et al.*, 1989; Place *et al.*, 1989), and may be the reason for differences in the rates of gastric evacuation of [^{14}C] tripalmitin between the two procellariiforms used in this study. The pylorus in the albatross is on the lateral surface of the ventriculus, and counts of ^{14}C in proventricular samples from the albatross were four orders of magnitude lower than corresponding counts for the White-chinned Petrel, indicating relatively rapid evacuation of lipids from the albatross stomachs. In the petrel, both the pylorus and the opening between the proventriculus and ventriculus are ventral, possibly facilitating more complete separation of lipid and aqueous dietary components in this species. In contrast to gastric evacuation rates of ^{14}C-labeled lipids, however, initial passage of [^{3}H] GTE through the entire gut is more rapid in the White-chinned Petrel than in the Sooty Albatross. Further comparative studies should improve our understanding of the mechanisms permitting differential gut passage rates of aqueous and lipid digesta within the procellariiforms

Our study demonstrates faster total gut evacuation rates of aqueous digesta than of lipids in both the White-chinned Petrel and the Sooty Albatross, but not in the Rockhopper Penguin, reflecting differing gastric evacuation rates of these dietary components between procellariiforms and penguins.

Gut evacuation in relation to foraging method

Fast gastrointestinal passage rates, hence rapid reduction of meal mass, should benefit flying seabirds more than the aquatic penguins, because the latter are neutrally buoyant in water whereas the former must expend energy in proportion to their total mass in order to stay aloft. The rapid evacuation of both water and lipids from Rockhopper Penguin guts was therefore contrary to our expectations. Passage rate per unit gut length is considerably faster in the penguin than it is in the two procellariiforms, because the former has the longest small intestine of the three species studied.

The differences we report in small intestine and total gut mass between the procellariiforms and the Rockhopper Penguin may be evidence of digestive adaptations to different foraging methods. Breeding seabirds are subject to energetic constraints involving the transport of food to their chicks (see Ricklefs, 1983). Penguins hunt while swimming, a foraging strategy that is more energetically expensive than gliding flight, but which greatly reduces weight-related constraints on gut and meal size. To meet their energy requirements, penguins must catch and process more food per unit time than must flying seabirds such as albatrosses and petrels. The latter have evolved an energetically economical mode of flight which enables them to forage over far larger areas than the penguins, but which reduces the accessibility of prey, because flying species forage largely in two dimensions, penguins in three. Meal size and gut length are weight-limited in flying seabirds (Sibly, 1981).

Comparison of the field metabolic rates of free-ranging penguins and albatrosses, measured using the doubly-labeled water technique, confirms the differences in the energetic costs of the two foraging strategies outlined above. Although there are no data for the three species used in our study, values for energy expenditure while foraging are available for the sympatric, congeneric Macaroni Penguin *Eudyptes chrysolophus*, and for three albatrosses. The values (kJ.[kg body mass]$^{-1}$.day^{-1}) are as follows: for the Macaroni Penguin, 1729.2 (Davis *et al.*, 1989),

the Grey-headed Albatross *Diomedea chrysostoma*, 681.7 (Costa and Prince, 1987); the Laysan Albatross *D. immutabilis*, 471.9 (Grant and Whittow, 1983; Nagy, 1987) and the Wandering Albatross *D. exulans*, 305.1 (Brown and Adams, 1986; Adams *et al.*, 1986). The relatively high energetic cost of foraging in penguins is demonstated by a comparison of the ratios of energy expenditure [at sea]:[at the nest] with corresponding values for albatrosses. The ratios are 4.88 for the penguin, and 2.33, 1.49 and 1.31 for the albatrosses respectively. The long intestine in the rockhoppper may thus be an adaptation to maximize gut capacity in order to meet the demands of an energetically costly foraging method.

Gastrointestinal transit time and moult in penguins

The Rockhopper Penguins used in our experiments had just completed moult, a process involving fasts of up to three weeks. The timing of the annual relief voyage to Marion Island rendered this complication unavoidable. Energy assimilation efficiencies for fish and crustaceans fed to post-moult Rockhopper Penguins, and to birds which were about to commence breeding and had not fasted, are statistically indistinguishable (S. Jackson, unpubl. data). The same is true for mean retention times of digesta in the guts of post-moult and pre-breeding individuals fed fish and squid. Mean gut retention times of crustaceans were, however, significantly longer in the post-moult birds than they were in the pre-breeding individuals (26.5 ± 0.2 and 20.2 ± 1.3 hours respectively, $U_{4,6} = 24$, $P \leq 0.01$, Chapter 3). If the post-moulting condition of the Rockhopper Penguins used in our study influenced excretion rates of lipids, the bias would reduce rather than enhance the differences between the procellariiforms and the penguin. Moreover, the carrier lipid excreted by penguins in the present study was largely hydrolyzed, indicating that the lipids were not simply passing undigested through the penguins' guts.

Gastrointestinal transit time and age

Domestic Fowl *Gallus domesticus* chicks exhibit faster gastrointestinal transit rates (Golian and Polin, 1984) than do adults or laying hens (Mateos and Sell, 1981;

Mateos *et al.*, 1982). In contrast, Sooty Albatross fledglings retain higher percentages of [^{3}H] GTE than do adults of the species, and cumulative lipid excretion curves for the lipid marker show pronounced differences between the two age groups. Initial excretion rates of [^{3}H] PEG were also slower in the fledgling albatrosses than in the adults. Roby *et al.* (1986) fed a ^{14}C-labeled wax ester, cetyl palmitate, to Least Auklet *Aethia pusilla* adults and chicks, and found that higher proportions of the wax ester were excreted by the adults than by the chicks. Pre-fledging seabirds may regularly fast for longer periods than their parents (Ricklefs, 1983). Slow transit of lipids may enable fledgling procellariiforms to eke out ingested meals between visits by their parents (Cheah and Hansen, 1970a). In addition, chicks may be accustomed to predigested meals, hence slow to digest undigested food such as that fed in our experiments.

Despite significantly slower gastric evacuation rates of the ^{14}C-labeled tripalmitin, Sooty Albatross fledglings did not assimilate this lipid any more efficiently than did their adult conspecifics. Energy assimilation efficiency is thought to be a function of the retention time of digesta in the gut (Sibly, 1981). Our data indicate that within-species variation in lipid passage rates may exert less influence over the rate of lipid hydrolysis and assimilation efficiency than do other factors, for instance, the nature and quantity of lipids present in the proventriculus at the time of ingestion (Place *et al.*, 1989; Place and Butler, subm.).

Neutral lipid assimilation efficiency

The petrel and albatross show high assimilation efficiencies of the ^{14}C-labeled triglyceride and the wax ester, similar to efficiencies of 96 - 99% estimated for assimilation of wax ester by Antarctic Prions *Pachyptila desolata*, Common and South Georgian Diving Petrels *Pelecanoides urinatrix* and *P. georgica* , and Least Auklets (Roby *et al.*, 1986); and of triglycerides and wax esters by Wilson's Storm-Petrels *Oceanites oceanicus* (Obst, 1986). Leach's Storm-Petrels assimilate up to 99% of ingested cetyl oleate, irrespective of the concentration of this wax ester in their diets (Place and Roby, 1986). In the current study, procellariiforms exhibit

high assimilation efficiencies of a saturated triglyceride (i.e. tripalmitin) even in the presence of a wax ester (i.e. cetyl oleate). The only other bird species that has been shown to assimilate wax esters, or to survive on pure wax diets, is the Lesser Honeyguide *Indicator minor* (Friedmann and Kern, 1956; Diamond and Place, 1988).

The low lipid assimilation efficiencies we report for Rockhopper Penguins are apparently associated with rapid evacuation of the ^{14}C-labeled neutral lipids from the gut. Interspecific differences in gastrointestinal passage rates of digesta between procellariiforms and penguins are probably linked to differences in foraging strategies.

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CHAPTER 6

CHITIN DIGESTION BY SEABIRDS

SUMMARY

Chitinase activity was detected in the stomach contents and gastric mucosae of five species of sub-Antarctic seabird (two procellariiforms and three penguins), but was not found in samples from Cape Gannets (*Morus capensis*). Enzyme activity (μg N-acetylglucosamine.mg protein^{-1}.h^{-1}) was strongest in samples from the gastric mucosa of the Sooty Albatross (*Phoebetria fusca*). No chitinase activity was detected in samples of the intestinal mucosae of the White-chinned Petrel (*Procellaria aequinoctialis*) and Sooty Albatross. A chitin balance study on these two procellariiforms and on Rockhopper and Gentoo penguins (*Eudyptes chrysocome* and *Pygoscelis papua*) revealed chitin assimilation efficiencies of between 34 and 95%. Chitin may provide seabirds with a supply of carbohydrates, but the major energetic advantage of enzymatic degradation of chitin is probably facilitation of digestion of soft prey tissues within the exoskeletons of crustacean prey.

INTRODUCTION

The chitin in crustacean exoskeletons has been suggested as a substantial source of potential energy for marine predators (Anderson *et al.*, 1978; Rehbein *et al.*, 1986). For this reason, chitin digestion in fish has been well studied (eg: Fange *et al.*, 1976). Endogenous chitinase synthesis by vertebrates was first reported by Jeuniaux (1961), and has since been documented in marine fish (Okutani, 1966; Rehbein *et al.*, 1986; Seiderer *et al.*, 1987), and in terrestrial mammals (Cornelius *et al.*, 1975; Cornelius *et al.*, 1976). The occurrence of chitinase in vertebrate digestive systems has been reviewed by Jeuniaux (1963) and more recently by Jeuniaux and Cornelius (1978), who reported gastric chitinase activity in eight species of partially insectivorous terrestrial birds. Chitinase activity was detected in the regurgitated pellets of a further seven raptor species by Leprince *et al.* (1979). The synthesis of chitinolytic enzymes (chitinase and chitobiase) is probably a primitive retained characteristic rather than a re-evolved one, and is prevalent among invertebrates (Jeuniaux, 1971). Chitinase production is not inducible in vertebrates that habitually eat food devoid of chitin, and synthesis of this enzyme persists in animals which normally feed on chitinous prey, once chitin has been excluded from their diets (Jeuniaux, 1971).

Seabirds such as those that feed on crustaceans in the Southern Ocean would benefit greatly from the ability to speedily penetrate the exoskeletal "defenses" of their prey. The present study tests the prediction that seabirds which habitually eat crustaceans, secrete chitinase. Although a study of microbial and vertebrate chitin degradation by crabeater seals and Adélie penguins is currently under way (Staley, 1986), I am not aware of any published data on the levels of chitinase activity in seabird guts. Here, I present the results of chitinase assays on stomach contents and tissue samples from five species of seabirds occurring in the Southern Ocean, and one species breeding on the west and south coasts of South Africa. There are marked interspecific differences in the major prey categories of these seabirds. I also report on a preliminary chitin balance study, giving assimilation efficiencies of

chitin for four of the five species of seabird found to have gastric chitinase activity.

METHODS

Sample collection

Samples were collected from the Sooty Albatross *Phoebetria fusca*, White-chinned Petrel *Procellaria aequinoctialis*, Rockhopper *Eudyptes chrysocome*, Gentoo *Pygoscelis papua* and King *Aptenodytes patagonicus* penguins, and from the Cape Gannet *Morus capensis*. Tissue samples were taken from the birds' stomach (proventriculus) linings using Olympus FB-15K E biopsy forceps passed through an Olympus GIF type P fiber-optic gastroscope with an Olympus CLE 4U/4E cold light source. This method did not necessitate killing the study animals (see Chapter 2). The samples were rinsed a minimum of five times with 67 mM phosphate buffer (pH 7.0), and immersed in a solution of 20 µl of PMSF (phenylmethanesulphonylfluoride, Merck, RSA) in 10 ml of phosphate buffer. The samples were frozen and stored at -20°C for one to two months before analysis. Two samples each were taken from four adult birds for each species, and samples were pooled for each species. Whole sections of stomach and intestinal tissue, including the mucosae, were dissected from one Sooty Albatross and two White-chinned Petrels that had been killed, and were frozen separately and stored at -20°C, each in 10 ml of a solution with the same proportions of phosphate buffer and PMSF as that used above.

Samples of stomach contents were removed from the proventriculi of three to six captive birds of each species, that had been fed a meal of Antarctic Krill *Euphausia superba* or prawn *Palaemon pacificus*, 12 h previously. The Cape Gannets were fed prawn, squid *Loligo vulgaris reynaudii* or pilchard *Sardinops ocellatus*, and samples for each of the three food types analyzed separately. Stomach contents were then withrawn through the oesophagus, using a tube attached to a 60 ml disposable syringe. It was necessary to introduce 30 ml of distilled water into each seabird's stomach to facilitate withdrawal of the sample.

Twenty µl of PMSF per 10 ml of stomach contents was added to each of the samples, which were stored as described above.

Enzyme assays

The tissue samples were thawed, crushed by hand over ice in 4 ml of phosphate buffer using glass tissue grinders, then centrifuged at 10 000 x g for 10 min and the supernatant decanted for assays. Samples of stomach contents were thawed and centrifuged in the same manner, but were not homogenized. All assays were performed in duplicate. The method used was end product measurement (Jeuniaux, 1966). Chitin substrate was prepared using the method of Reichenbach and Dworkin (1981). Samples were incubated on a shaker at room temparature, with 1.5 ml enzyme solution and 600 µl of chitin slurry. One hundred µl of toluene was added every 24 h to inhibit bacterial activity. At 0, 4, 24, 48 and 72/96 h after the start of incubation, 250 µl aliquots of the solution were removed and assayed for the end-product B-1,4 linked N-acetylglucosamine (NAG). The samples were read on a Beckman DU-40 spectrophotometer at wavelength 585 nm, and absorbance was converted to chitinolytic activity (µg NAG) using the equation:

$$y = 9.43\, x^{0.87}\ (N = 16,\ r^2 = 0.99)$$

where y is µg NAG and x is absorbance at 585 nm. The equation was calculated from a dilution series with standard solutions of NAG (Sigma) in phosphate buffer.

Protein content of the samples was determined using the method of Lowry *et al.* (1951), and final enzyme activity values were expressed as µg NAG produced per mg protein per hour.

Chitin balance

Assimilation efficiencies of chitin were determined for the Sooty Albatross, White-chinned Petrel, and Rockhopper and Gentoo penguins, all of which showed gastric chitinase activity. Five birds of each species were fasted for 48 h before receiving a krill meal of wet mass 6 - 8% of bird body mass. The birds were then

fasted for a further 48 h, and all faeces voided since feeding collected separately for each bird. Faeces and samples of food were dried at 45°C for five days, and then weighed and ground finely in an electric coffee grinder. The chitin content of food and faeces samples was determined using the method for crude fiber determination in animal feeds described by Horwitz *et al.* (1975). The fat was extracted from dried, ground samples using diethyl ether. Each fat-free sample was then transferred to a 500-ml conical flask, to which was added exactly 1 g of prepared asbestos (see below), 1 drop of Antifoam B Emulsion (diluted 1:4 with water, Dow Corning Corp., USA) and 200 ml boiling 1.25% H_2SO_4 (0.225 ± 0.005N). This solution was boiled for exactly 30 min, then filtered through cotton cloth stretched over a Buchner funnel. Material adhering to the sides of the flask was rinsed into the funnel with 50 - 75 ml of boiling water, followed by a further three 50-ml rinses of water. Once sucked dry, the cloth and residue were removed from the funnel, and the residue scraped and rinsed back into the conical flask with 200 ml 1.25% NaOH (0.313 ± 0.005N, free of Na_2CO_3). This solution was then boiled for exactly 30 min, and the residue once again scraped and rinsed back into the funnel using a further 200 ml of NaOH. The residue was then washed with 25 ml boiling 1.25% H_2SO_4, six 50 ml rinses of water, and 25 ml 95% ethyl alcohol. The then dry residue (chitin plus asbestos) was separated from the cloth, transferred to a porcelain ashing dish, dried at 130°C for 2 h, cooled in a desiccator, and weighed, before being ashed at 600°C for 30 min, cooled and reweighed. The chitin content of the sample was estimated as the mass loss of the residue on ashing, minus the mass loss (usually < 1 mg) of 1 g of pure prepared asbestos treated with acid and alkali and ashed in the same manner as the sample. Chitin content was expressed as a percentage of the dry matter of the original sample. Asbestos (Gooch grade) was prepared by boiling in 5% NaOH for 15 min, filtering through sintered glass funnel by suction, washing with boiling water, refiltering, boiling in HCl (diluted 1:3 with water), refiltering, and finally ashing at 600°C for 2 h. All water used was distilled.

Assimilation efficiencies of chitin for individual birds were calculated as

percentages using the formula:

((chitin in - chitin out)/ chitin in) x 100

where "chitin in" and "chitin out" are total dry masses (g) of chitin in the food and the faeces respectively.

RESULTS AND DISCUSSION

Enzyme activity in relation to natural diet

Kerry (1969) reported very low levels of disaccaridase activity in five Southern Ocean seabirds; Rockhopper, Gentoo and King penguins, and the Brown Skua (*Stercorarius skua*) and Kelp Gull (*Larus dominicanus*). The chitinase activities presented in Table 6.1, are, to my knowledge, the first evidence of substantial carbohydrase activity in any seabird. Chitinase secretion may be restricted to seabirds preying naturally on crustaceans. Of the five species of Southern Ocean seabird sampled in the present study, all but the King Penguin showed chitinase activity in the gastric mucosa, at similar levels despite wide differences in the proportion of crustaceans in their natural diets (Table 6.2). The absence of chitinase activity in the stomach tissues of King Penguins may reflect the almost complete absence of crustaceans in their diet (< 1% by numbers, Adams and Klages, 1987), and the same may be true for the Cape Gannet (Berruti and Colclough, 1987).

In view of the fact that the ability to secrete chitinase is shared by many invertebrates (Jeuniaux, 1971), it seems likely that synthesis of this enzyme is a retained characteristic widespread among high-latitude seabirds because of the advantages it confers on opportunistic marine predators feeding in waters rich in crustacea.

Activity levels of chitinase in the seabirds studied (Table 6.2) are of the same order of magnitude as those reported for Antarctic fish stomachs (Rehbein *et al.*,

Table 6.1. Chitinase activities in the stomachs and intestines of six species of seabird (μg N-acetylglucosamine. mg protein^{-1}. h^{-1}). Unless otherwise indicated, activities are from the proventriculus. Sampling methods: B, sample taken using biopsy forceps; SC, stomach contents sampled. D, tissue dissected from dead bird. Negative values are assumed to indicate absence of chitinase activity.

Species	Sampling method	Incubation time				Mean ± 1 S.E.
		4h	24h	48h	72/96h	
Rockhopper Penguin	B	—	0.55	1.75	0.38	0.90 ± 0.75
	SC	-11.61	-2.04	-0.31	-0.75	-3.68 ± 5.34
	SC	-0.65	-0.16	-0.33	0.15	-0.32 ± 0.24
Gentoo Penguin	B	—	0.65	0.92	0.71	0.75 ± 0.14
	SC	—	-0.28	-0.03	-0.01	-0.11 ± 0.15
King Penguin	B	—	-0.16	0.09	0.05	-0.01 ± 0.14
	SC	—	-0.05	-0.001	0.001	-0.02 ± 0.03
Sooty Albatross	D	—	0.37	0.30	—	0.34
	B	—	0.31	1.57	1.41	1.09 ± 0.69
	SC	4.25	4.49	4.71	4.20	4.41 ± 0.24
	SC	6.78	-2.52	12.84	11.58	7.17 ± 6.97
(intestine)	D	—	-0.11	-0.07	—	-0.09
White-chinned Petrel	D	—	0.19	0.09	—	0.14
(intestine)	D	—	-0.01	-0.07	—	-0.04
Cape Gannet	SC	—	-0.10	-0.16	—	-0.13
	SC	—	-0.06	-0.05	—	-0.05
	SC	—	0.52	-0.06	—	0.23

Table 6.2. Predominant prey types of seabirds studied (less frequently eaten prey in parentheses).

Species	Predominant prey	Reference
Rockhopper Penguin *Eudyptes chrysocome*	crustacea (fish)	Brown & Klages (1987)
Gentoo Penguin *Pygoscelis papua*	fish (crustacea)	Adams & Wilson (1987)
King Penguin *Aptenodytes patagonicus*	fish (squid)	Adams & Klages (1987) Croxall & Prince (1980)
Sooty Albatross* *Phoebetria* sp.	squid	Thomas (1982)
White-chinned Petrel *Procellaria aequinoctialis*	fish (squid, crustacea)	Jackson (1988) Berruti (unpubl. data)
Cape Gannet *Morus capensis*	fish	Berruti & Colclough (1987)

* Diet data available only for Light-mantled Sooty Albatross *Phoebetria palpebrata*

1986), and are very similar to corresponding levels detected in anchovy guts (Seiderer *et al.*, 1987). Unlike the anchovy, however, there appears to be no intestinal chitinase activity in the White-chinned Petrel and the Sooty Albatross, the only seabirds from which intestinal tissue was sampled.

The biopsy method of tissue sampling yielded positive assay results for Rockhopper and Gentoo penguins, whereas stomach contents sampled within minutes of the biopsies and from the same individual birds, yielded negative results. This suggests that the chitinase was secreted by the birds' gastric mucosae, rather than produced by bacteria in the stomach lumen. In addition, all tissue samples were thoroughly rinsed before freezing, and assays were carried out in the presence of toluene, which inhibits bacterial activity. Therefore, the chitinase detected in Rockhopper and Gentoo penguins is probably of endogenous rather than microbial origin. However, the chitinase detected in samples of stomach contents from Sooty Albatrosses fed squid, and from Sooty Albatrosses and Cape Gannets fed prawns, may be of microbial origin, or may have originated from the prey (see ZoBell and Rittenberg, 1937, for a record of chitinase activity in squid). The positive results yielded by tissue samples taken with biopsy forceps indicate that this non-lethal sampling method may prove useful for workers wishing to avoid killing their study animals (see also Chapter 2).

Repeated assays at different ambient temparatures may reveal that bird body temparature (39°C) is closer to the optimum for action of the chitinase detected in this study, than is the temparature used in this study.

Chitinolytic bacteria in seabird guts.

Chitinolytic bacteria have been implicated in the digestion of chitin in fish (Okutani, 1966, Goodrich and Morita, 1977). Soucek and Mushin (1970) isolated gram-negative bacteria from the guts of eight species of penguin and two species of skua. Although *Escherichia coli* predominated, seven out of 95 Adélie Penguins and one out of 10 Rockhopper Penguins sampled had *Enterobacter* in their intestines. *Enterobacter* is known to produce large amounts of chitinase (Monreal

and Reese, 1969). There is thus a strong possibility that bacteria aid in the breakdown of chitin in seabird hindguts. The acidity of seabird stomach contents (pH 0.50 - 2.75, van Dobben, 1952, Place *et al.*, 1986) may preclude bacterial action in the foregut.

Chitin assimilation efficiency

The krill fed to experimental birds contained 2.9 ± 0.4% chitin (dry matter, see Chapter 4 for water content). Chitin assimilation efficiencies (± 1 S.D.) were 34.49 ± 15.37, 39.31 ± 4.94, 52.77 ± 37.64, and 45.29 ± 5.63 for the Sooty Albatross, White-chinned Petrel, Rockhopper Penguin and Gentoo Penguin, respectively. These species are thus able to assimilate a substantial proportion of the chitin that they ingest. Chitin is probably not a significant source of energy to seabirds, because crustaceans such as krill comprise only a small proportion of chitin (between 2.1 and 2.9%, dry mass, Clarke, 1980; Chapter 4). Assuming that chitin has the same energy value (17.9 $kJ.g^{-1}$ dry mass) as whole krill (Karasov, in press), seabirds able to assimilate 40 - 90% of ingested chitin would derive a maximum of between 1.2 and 2.7% of their total energy gain from the chitin fraction of each meal. The major benefit to seabirds of chitinase synthesis is thus probably enhanced efficiency of digestion of soft prey tissues within the exoskeletons of their crustacean prey, rather than elevated energy assimilation efficiency.

The possibility that retention of chitinous material in the birds' guts resulted in overestimation of chitin assimilation efficiencies, should be tested by feeding experiments using radio-labelled chitin.

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CHAPTER 7

ECOLOGICAL AND PHYSIOLOGICAL CORRELATES OF SEABIRD DIGESTIVE PROCESSES

SUMMARY OF FINDINGS

1. *In vitro* standards show that of the prey types used in the feeding experiments, fish is the most digestible, followed by squid, krill and prawns. Previously-frozen samples are digested more rapidly than is fresh tissue, but the ranking of prey digestibility is not influenced by this treatment.

2. Fiber-optic endoscopes have potential application in studies of gastric digestion in marine vertebrate predators. Biopsy sampling of the gastric mucosa is a non-lethal technique that may be used in enzyme studies. For *in vivo* digestion studies, serial inspection using a gastroscope is less stressful than recovery of stomach contents by sequential stomach-pumping, but yields qualitative rather than quantitative information on digestion rates.

3. Feeding experiments using five seabird species indicate that gut passage rates of squid may be faster in seabirds which eat squid in the wild, than in the Cape Gannet, a specialized piscivore. Mean retention times of solid digesta are significantly correlated with foraging trip duration, and with gut length. Gut length and volume in turn scale with body mass. Gut passage rates may reflect physiological and morphological specialization to different foraging methods.

4. Assimilation efficiencies of various dietary components by seven seabird species show no clear trends in relation to natural diet, and are not predictable purely on the basis of food composition. Inter- and intraspecific variability are high. Energy assimilation efficiency may be a constant independent of food type, with mean values converging on 75% for all species.

5. Unlike most terrestrial vertebrates, seabirds are able to digest wax esters, compounds important in marine food webs. Procellariiforms exhibit unique gastric adaptations facilitating extended foraging trips and efficient transport of food to their young, both important advantages for predators exploiting patchy and unpredictable food resources.

6. Seabirds which naturally feed on crustaceans secrete the specific enzyme chitinase from their gastric mucosae. Chemical digestion of chitinous exoskeletons

of crustaceans presumably enhances efficiency of digestion of the soft tissues of this prey type. The ability to secrete chitinase is probably a retained ancestral trait rather than a newly evolved one, that has probably been lost by seabirds that do not prey on crustaceans.

INTRODUCTION

Body size mediates animals' interactions with their environment (Peters, 1983; Schmidt-Nielson, 1984). If one assumes that animal design is optimized (Schmidt-Nielsen, 1984) then the body size of each species represents a compromise solution to the interaction of all the abiotic and biotic influences on that animal. In particular, physical properties related to body dimensions limit the possible range of solutions to animal design problems involving metabolic rate (hence energy requirements; Nagy, 1987) and locomotion (hence foraging; Pennycuick *et al.*, 1984). In this chapter, I attempt to link the structural and functional digestive adaptations described in previous chapters by means of allometry. The approach is designed to clarify the relationship between environmental factors and the digestive processes of seabirds. Causal relationships are not necessarily implicit in the correlations between structural and functional parameters that are presented here. Rather, the correlations contribute to an understanding of the interplay between these parameters.

The assumption that animals are structured to meet, but not exceed, their functional requirements (Schmidt-Nielsen, 1984) has been proposed and tested for mammalian respiratory systems by Taylor and Weibel (1981) and Weibel *et al.* (1981). These authors concluded *inter alia* that understanding of the relationship between mass-dependent structural parameters and oxygen requirements is best achieved by experimental manipulation of functional loads on the respiratory systems of animals of different body sizes.

Because gut tissue carries high maintenance costs relative to muscle (Schmidt-Nielsen, 1984), and because digestion of seabird prey may cost nearly one quarter of the energy value of the food itself (Ricklefs, 1974), one might assume that seabird guts and digestive processes will not be maintained at sizes and levels of efficiency that more than cater for the energy needs of the birds. However, seabird digestive systems do not lend themselves to experimental manipulation in the same way that mammalian respiratory sysems do. Weibel *et al.* (1981) measured respiratory

parameters at maximum performance levels in mammals trained to run on treadmills, but the immediate demands on digestive systems cannot be forced to maxima in short term experiments such as those used in this study. Animals store ingested energy, and the previous plane of nutrition influences avian gut size, hence capacity for processing food (Leopold, 1953; Moss, 1974). Scaling of gut size and digestive and foraging parameters to match metabolic energy demands (which scale with body mass$^{0.714}$, see below) may thus be masked by birds' physiological states prior to the experiments. The experimental results used in the following discussion were obtained primarily from adult birds in the immediate post-breeding period, but the White-chinned Petrel fledglings and pre-breeding adult Rockhopper Penguins are exceptions (results from the post-moulting Rockhopper Penguins and fledgling Sooty Albatrosses are not included). Ideally, discussions such as the one that follows should be supported by data from birds of identical physiological status. In the interests of maintaining a base of species wide enough for meaningful discussion, I decided not to discard data for the two species in question. The numerical relationships given below are thus intended as a framework for discussion of the adaptive significance of avian digestive process, and are open to refinement by the addition of more data.

The value of allometry in promoting understanding of physiological (eg: Lindstedt and Calder, 1976; Calder, 1981; Karasov, in press) and ecological (eg: Calder, 1983; Peters, 1983) adaptations is well recognized. Brown *et al.* (1978) provide a comprehensive synthesis of the ecological and evolutionary significance of body size in nectarivorous birds. However, an allometric approach has not previously been applied to studies of seabird digestion.

DISCUSSION

Fig. 7.1 is a schematic representation of the structural and functional allometric relationships described in Chapters 3 and 4. Ecological parameters that have immediate bearing on these relationships have been included. In the section that

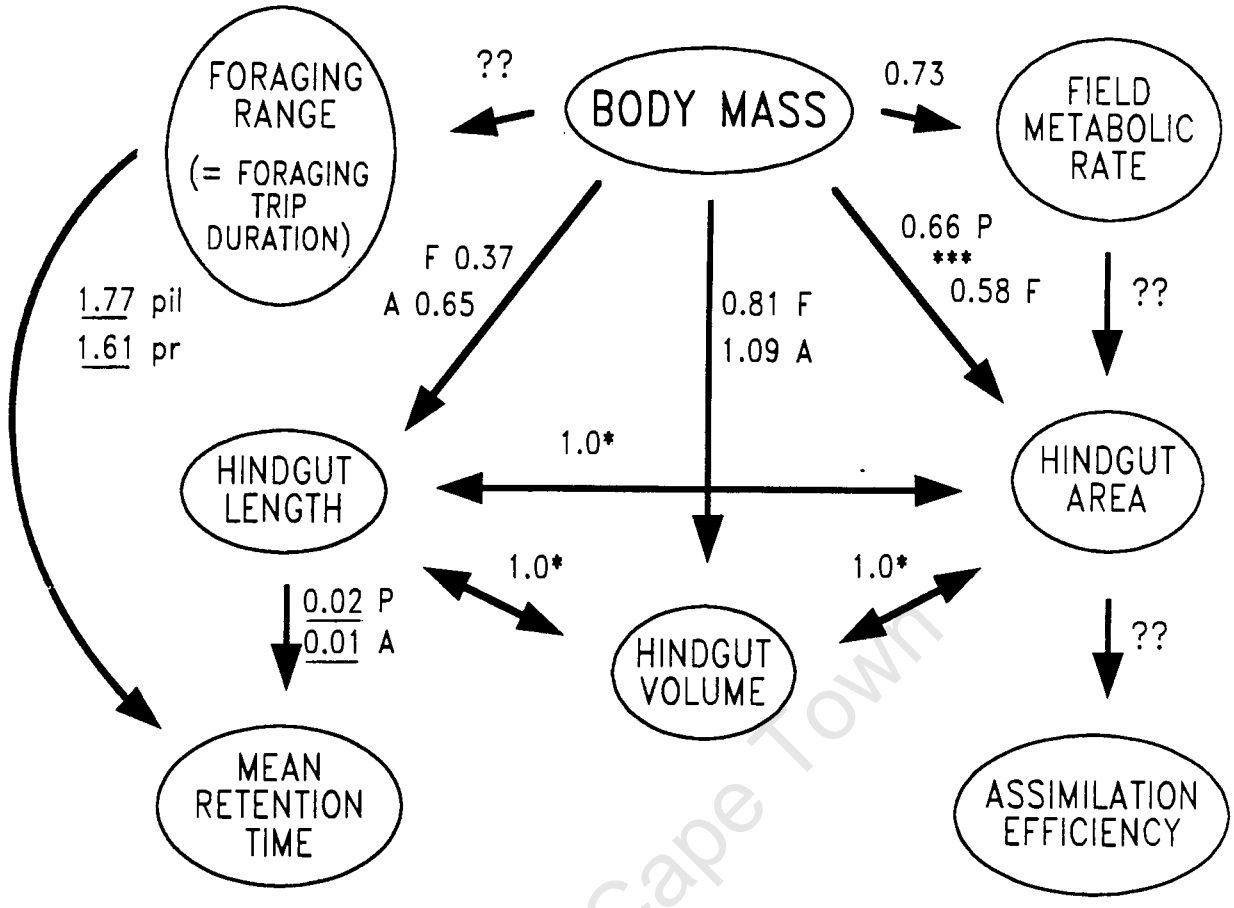

Figure 7.1. Schematic representation of the relationships between digestive and related foraging parameters, and body size. Values next to arrows are the x-exponents. Underlined exponents denote linear regressions, all others being logarithmic. ***: Significant difference between both slopes and y-intercepts of two regressions, $P < 0.005$. Hindgut volume and surface area were calculated using measurements of hindgut length (see Chapter 3), resulting in exponents of one for these regressions.

Equations:

$T(pil) = 8.66 + 1.77.FTD$, $r_3 = 0.97$, $P < 0.01$.
$T(prawn) = 15.59 + 1.61.FTD$, $r_3 = 0.90$, $P < 0.05$.
$T(pil) = 5.12 + 0.02.HL$, $r_1 = 0.999$, $P < 0.025$ (penguins only)
$T(prawn) = 12.61 + 0{,}01.HL$, $r_3 = 0.81$, $P < 0.05$ (all species studied)
$\log HL = 0.11 + 0.65.\log BM$, $r_{11} = 0.84$, $P < 0.001$ (all species)
$\log HL = 0.91 + 0.37.\log BM$, $r_7 = 0.76$, $P < 0.02$ (flying species only)
$\log HV = -1.87 + 1.09.\log BM$, $r_{10} = 0.93$, $P < 0.001$ (all species studied)
$\log HV = -1.06 + 0.81.\log BM$, $r_6 = 0.87$, $P < 0.005$ (flying species only)
$\log HA = -0.35 + 0.88.\log BM$, $r_{11} = 0.93$, $P < 0.001$ (all species studied)
$\log HA = 0.49 + 0.58.\log BM$, $r_7 = 0.88$, $P < 0.002$ (flying species only)
$\log HA = 0.58 + 0.66.\log BM$, $r_2 = 0.95$, $P < 0.05$ (penguins only)
$\log FMR = 0.90 + 0.73.\log BM$, $r_{12} = 0.95$, $P < 0.001$.

A: all species, F: flying species only, P: penguins only. pil: mean retention time calculated for pilchard, pr: mean retention time calculated for prawn. T(pil): mean retention time (h) of pilchard (calculated 30h after feeding), T(prawn): mean retention time (h) of prawn (calculated 48h after feeding), FTD: foraging trip duration (days), HL: hindgut length (cm), BM: body mass (g), HV: hindgut volume (cm^3), HA: hindgut planar surface area (cm^2), FMR: field metabolic rate ($kJ.d^{-1}$).

Note: Y-intercepts given here for equations relating gut dimensions to body mass are different to those given in Chapters 3 and 4, because the units used in chapters 3 and 4 were kg.

follows, each factor will be discussed in turn. For two reasons, many of the ecological parameters drawn into the discussion relate specifically to breeding seabirds. Firstly, the feeding of chicks places sometimes severe energetic demands on the foraging capabilities of parent birds, and adaptations maximizing energy returns from foraging presumably evolved to cope with this period of stress. It is therefore informative to relate digestive processes to the foraging ecology of breeding birds. Secondly and more pragmatically, seabirds are at their most accessible when breeding, and much of the published data (for instance, on foraging range and metabolic rate) relates to breeding adults constrained to return to feed their young.

Hindgut length

Hindgut length scales with body mass$^{0.5}$ in Spanish passerines (Herrera, 1986), and with (body mass$^{0.32}$) in tetraonids (data from Leopold, 1953; cited by Karasov, in press). Hindgut length scales significantly with body mass$^{0.37}$ for the subset of flying seabirds which I studied, and is the primary determinant of mean gut retention time (Chapter 3). In a discussion of life-history parameters and physiological time, Lindstedt and Calder (1976) predicted that digestion rate should be dependent on animal body size, and this is indeed true for herbivorous mammals (body mass$^{0.28}$, Demment, 1982) and birds (body mass$^{0.22}$, Karasov, in press). The data used by Karasov (in press) were for birds feeding on seeds, fruit, foliage or arthropods, and he stressed the need for comparable data from birds eating vertebrate prey. Chapter 3 of this thesis provides such data, revealing that for the seabirds studied, body mass exerts an indirect influence on mean retention time of digesta.

But how does gut retention time in seabirds influence their foraging abilities? Sibly (1981) stated that "a birds' digestive strategy should minimize the weight carried". Ricklefs (1983) tested the truth of this for seabirds by quantifying the energetic costs to parent birds of transporting food to their chicks. Using equations developed by Pennycuick (1969, 1975), Ricklefs estimated the power requirements

of flight as a function of, *inter alia*, body mass and wing area for Sooty Terns *Sterna fuscata*. He found that the energy cost of transporting a meal for four hours was trivial in comparison with the energy value of the meal itself. He suggested that the ability to lift loads off the water might limit meal size in seabirds, rather than the energetic cost of transporting meals once on the wing. Pennycuick *et al.* (1984) use empirical estimates of delivered power relative to foraging radius in different-sized procellariiforms to suggest that "pay load" (maximum meal size) becomes proportionally less as body mass increases, implying, for example, that an albatross weighing more than 12kg would not be able to carry enough food to meet its own energy requirements plus those of its chick.

Because hindgut volume in flying seabirds scales with body mass$^{0.81}$ (Chapter 3), larger seabirds have proportionally shorter hindguts in relation to their body mass. Stomach volume scales with body mass$^{1.54}$ in flying seabirds, and with body mass$^{1.45}$ in penguins (Fig. 7.2). For flying seabirds, minimization of mass carried increases in importance parallel with seabird size, and the difference in scaling exponents may reflect a trade-off with gastric capacity increasing at the expense of hindgut size. Maximization of stomach capacity may be adaptive, because the stomach acts both as a "fuel tank" for adult seabirds, and as a storage organ for transporting food to chicks. Stomach oil formation in procellariiforms (Chapter 5) is another expression of the need to maximize the energy value of the stomach contents under weight-limited conditions. Comparison with penguins lends indirect support to this conclusion. Penguins design is not weight-limited, and the difference between scaling exponents for stomach (1.45, Fig. 7.2) and hindgut volume (1.27, Chapter 3) is less pronounced in this group. The regression of hindgut volume against body mass is not significant. In the absence of a broader data base, the above hypothesis remains speculative. Separate correlations for flying seabirds and for penguins yielding separate body mass-exponents for gut passage rate and gut length in these two groups would greatly contribute to our understanding of the role of flight-related constraints in seabird foraging ecology.

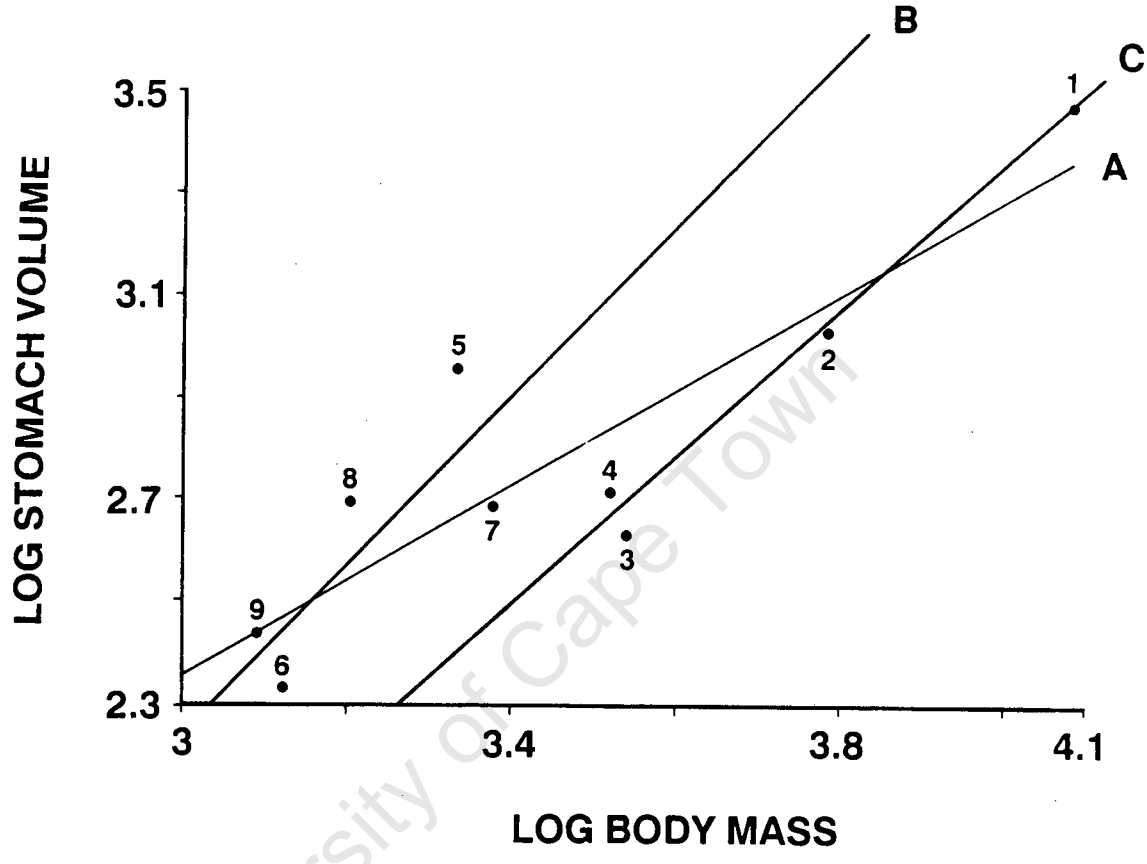

Figure 7.2. Stomach volume (cm^3 = g fresh mass of maximum meal size reported for birds in the wild) expressed as a function of body mass (g) for nine seabird species. Sources of stomach capacity estimates given in parentheses after each species name. 1: King Penguin *Aptenodytes patagonicus* (Adams and Klages, 1987), 2: Gentoo Penguin *Pygoscelis papua* (Croxall and Prince, 1980), 3: Rockhopper Penguin *Eudyptes chrysocome* (Brown and Klages, 1987), 4: Jackass Penguin *Spheniscus demersus* (Wilson *et al.*, subm.), 5: Sooty Albatross *Phoebetria fusca* (value assumed equal to that reported by Thomas, 1982, for Light-mantled Sooty Albatross *Phoebetria palpebrata*), 6: White-chinned Petrel *Procellaria aequinoctialis* (Berruti *et al.*, 1985), 7: Cape Gannet *Morus capensis* (N.J. Adams, pers. comm.), 8: White-breasted Cormorant *Phalacrocorax carbo* (van Dobben, 1952), 9: Cape Cormorant *P. capensis* (Davies, 1956).

A (All species): Log SC = -0.45 + 0.93 Log BM, $r_7 = 0.88$, $P < 0.002$, where SC = stomach capacity (cm^3).

B (Flying species only): Log SC = -2.34 = 1.54 Log BM, $r_3 = 0.80$, $P > 0.10$.

C (Penguins only): Log SC = -2.45 + 1.45 Log BM, $r_2 = 0.999$, $P < 0.001$.

Foraging range

Interpretation of the correlation between foraging trip duration and mean retention times of digesta (Chapter 3) is confounded by inclusion of both penguins and flying species in this regression. Within the penguins, the identical ranking by species of foraging trip durations and gut lengths (Chapter 3) may be coincidental, or gut length may be influenced the different energy demands imposed by differences in foraging range of these three species: Gentoo Penguins at Marion forage closest inshore and King Penguins farthest offshore, with Rockhopper Penguins foraging at intermediate distances (Adams and Brown, 1989). Davis *et al.* (1989) found that mass-specific daily energy expenditure in the inshore-foraging Gentoo Penguins at South Georgia was 29% lower than in Macaroni Penguins *Eudyptes chrysolophus*, which forage farther offshore and for longer periods. When corrected for the scaling of metabolic rate with body mass, the difference dropped to 17%, still a substantial figure. A similar difference might be expected between the energy requirements of Rockhopper and Gentoo penguins, with the longer guts of the former representing an adaptation for processing greater quantities of food to satisfy higher energy demands (Sibly, 1981). Gut capacity is more fully discussed in relation to metabolic demands and assimilation efficiency in the following section.

The relationship between foraging radius and body mass in procellariiforms is not linear (Pennycuick *et al.*, 1984), but maximum foraging radius may be set by size-related energetic constraints. Mass-specific metabolic rate in extremely small seabirds (storm-petrels) is high, and parent birds must therefore spend a greater proportion of their time during each foraging trip feeding, reducing available travelling time. Below a certain hypothetical size, seabirds cannot forage over a great enough area to meet both their own energy needs, and those of their chicks. The upper limits to body size of flying seabirds, as discussed above, may be set by the load that can be lifted.

Does gut capacity limit body size in seabirds?

A review of published data by Kirkwood (1983) indicated that there exists an upper limit to metabolizable energy intake in birds and mammals, but he did not suggest a reason for this limit. Mass-specific energy intake may be restricted ultimately by mass-specific gut capacity. Gut volume and metabolic rate scale with different exponents of body mass in ruminants (1.05 and 0.75 respectively), raising questions about the limits to body size in herbivores (Demment, 1983; Demment and van Soest, 1985). Large animals have an energetic advantage, because they have a greater gut capacity in proportion to their metabolic needs than do smaller species. The theoretical lower body size limit within this group is thus the mass below which gut capacity is insufficient to meet the animal's energy requirements by the relatively slow process of fermentation of vegetable matter. Seabirds feed on food of far higher energy density than do herbivores, and consequently have to process lower volumes of digesta to meet their energy needs. Both stomach and total gut capacity scale with higher exponents of body mass (0.93 and 1.01 respectively, Figs. 7.2 and 7.3, see below for calculation of these parameters) than does field metabolic rate (0.73, see Fig. 7.4). Large size therefore confers the same advantage on seabirds as it does on herbivores, but the lower limits to seabird body size may be influenced by gut capacity. Comparison of the y-intercepts for the relationships between seabird body size and gut capacity should yield insight into these limits, and for this purpose FMR and gut capacity must be expressed in the same terms.

The most convenient currency to use is metabolizable energy in grams (dry mass) of food. Data from Nagy (1987) on the energy requirements of ten species of seabird, were combined with recently-published values for an additional four species (Costa and Prince, 1987; Davis *et al.*, 1989; Adams *et al.*, subm.) to derive the equation

$FMR = 0.524\ BM^{0.730}$, equivalent to:

$\log FMR = -0.280 + 0.730 \log BM$

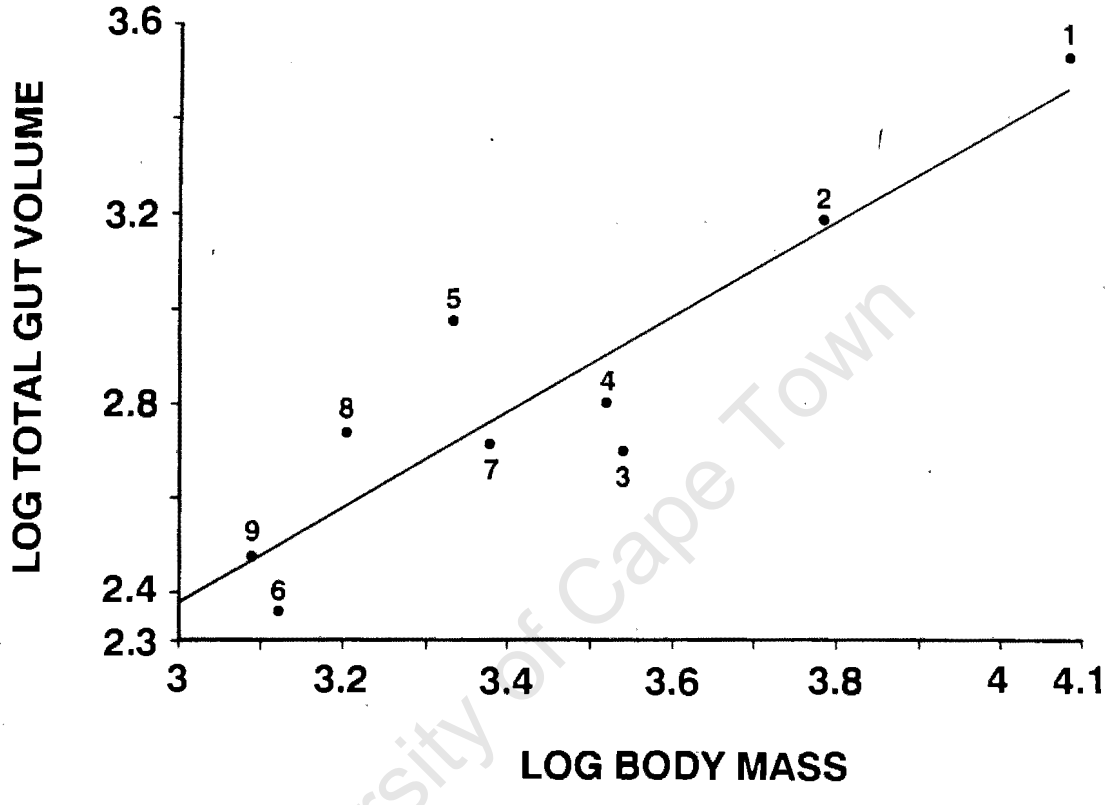

Figure 7.3. Total gut volume (stomach volume plus hindgut volume, cm^3) as a function of body mass (g) for the nine species depicted in Fig. 7.2.
Log GC = -0.66 + 1.01.log BM, r = 0.91, P < 0.001 for 7 df, where GC = total gut capacity (cm^3).

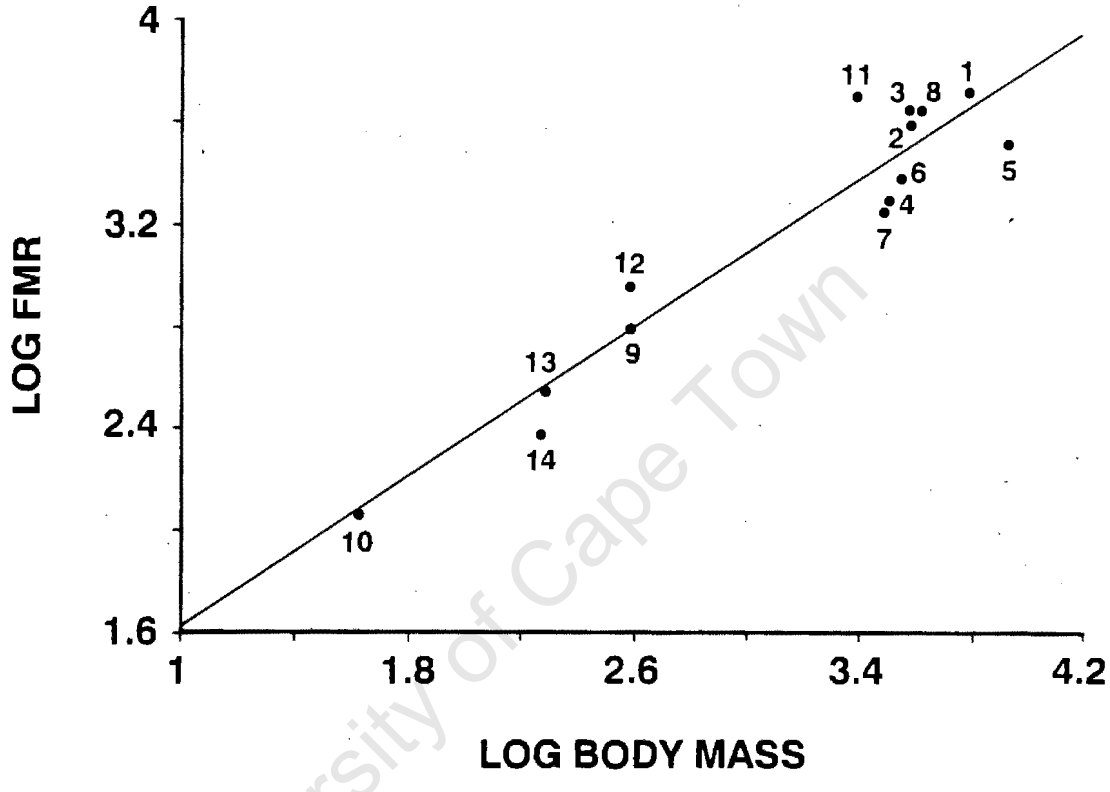

Figure 7.4. Field metabolic rate as a function of body mass for 14 species of seabird. Sources: Nagy (1987), Costa and Prince (1987), Davis *et al.* (1989), Adams *et al.* (subm.). 1: Gentoo Penguin *Pygoscelis papua*, 2: Adélie Penguin *P. adeliae*, 3: Macaroni Penguin *Eudyptes chrysolophus*, 4: Jackass Penguin *Spheniscus demersus*, 5: Wandering Albatross *Diomedea exulans*, 6: Grey-headed Albatross *D. chrysostoma*, 7: Laysan Albatross *D. immutabilis* 8: Southern Giant Petrel *Macronectes giganteus*, 9: Wedge-tailed Shearwater *Puffinus pacificus*, 10: Wilson's Storm-petrel *Oceanites oceanicus*, 11: Cape Gannet *Morus capensis*, 12: Kittiwake *Rissa tridactyla*, 13: Common (Brown) Noddy *Anous stolidus*, 14: Sooty Tern *Sterna fuscata*. Equation for the regression given in Fig. 7.1.

where FMR is field metabolic rate (g metabolizable dry food per day), and BM is body mass (kg). The scaling exponent of the new equation is slightly higher than Nagys' (1987) value of 0.704, but is identical to the value for basal metabolic rate in non-passerine birds given by Kendeigh *et al.* (1977). It does not differ substantially from resting metabolic rate values for incubating petrels and penguins calculated using oxygen consumption and mass loss-data (Croxall, 1982). However, the same techniques used on moulting penguins yield different exponents (0.77 to 0.83) for the scaling of energy expenditure to body mass (Adams and Brown, in press). To ensure validity of interspecific comparisons (LaBarbera, 1989), a single mean value for the body mass and FMR of each species was used. Only FMRs measured using the doubly-labelled water technique were used, and values in $kJ.d^{-1}$ were converted to grams of dry metabolizable matter using a value of $15.05kJ.g^{-1}$ (dry mass) for the mean metabolizable energy content of fish, squid and krill, estimated as the product of mean MEC of these foods and their energy density in $kJ.g^{-1}$ (Chapter 4). This value differs slightly from the one used by Nagy (1987) ($16.2\ kJ.g^{-1}$), and was selected to facilitate comparison with estimates of gut capacity.

Seabird stomach capacity was assumed to be the maximum meal mass reported for each species in the wild (see Fig. 7.2 for published sources). This figure (g fresh mass) was converted to dry metabolizable matter using a conversion factor (0.1875) equal to the product of the mean metabolizable energy coefficient (0.75) determined for three food types (Chapter 4), and the mean dry matter content of these foods (0.25, Chapter 4).

The results of this exercise are shown in Fig. 7.5. As a function of a hypothetical range of seabird body masses, line (A) represents daily energy requirements ($g.d^{-1}$), and line (B) represents the energy value of one stomach load (g dry metabolizable food). Line (C) is the energy value of two stomach loads, line (D) that of three loads, and so on up to five stomach loads. The implication of the intercept of, say, lines (A) and (B), is that a hypothetical seabird of mass 19.95 kg would have to fill its stomach to capacity once per day to meet its daily energy requirements.

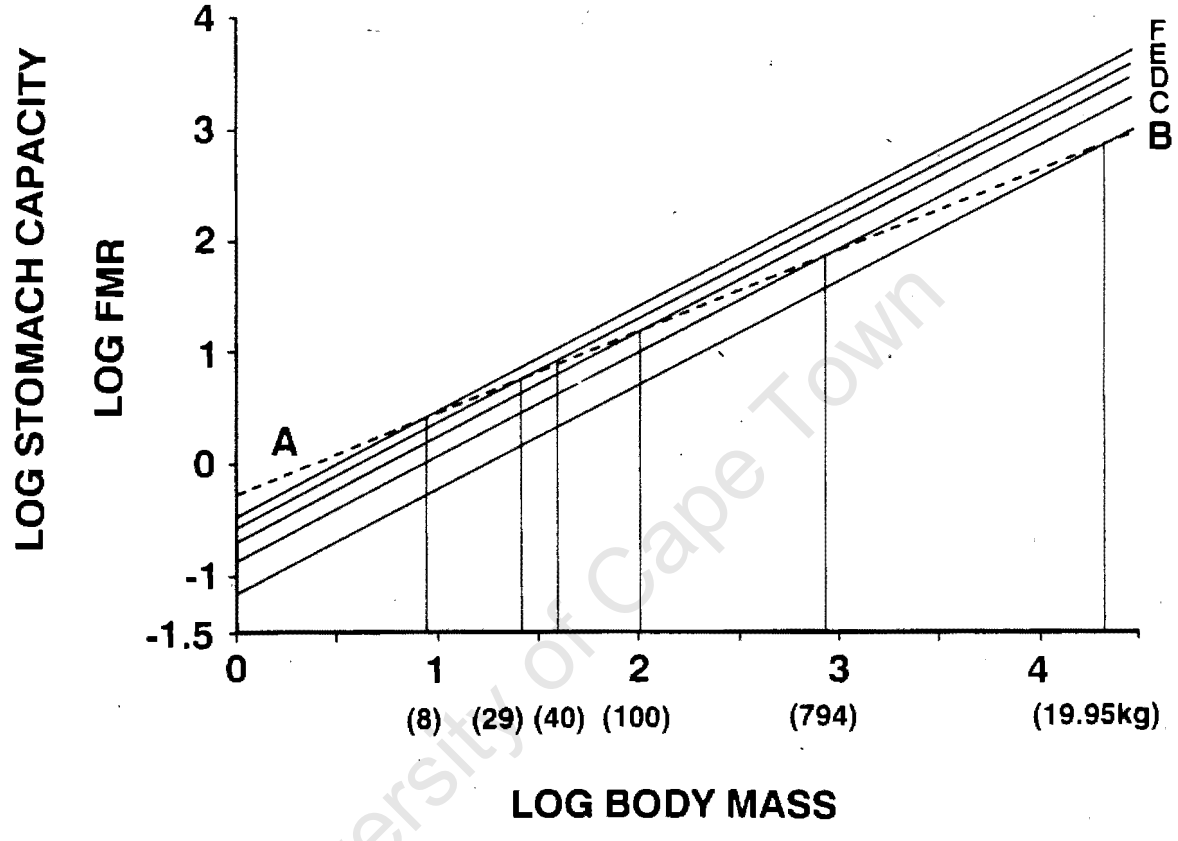

Figure 7.5. Field metabolic rate (g metabolizable dry matter.d^{-1}) and stomach capacity (g metabolizable dry matter) as functions of body mass. Data obtained from Figs. 7.2 and 7.4. A: FMR, B: stomach capacity, C, D, E, and F: 2 x, 3 x, 4 x, and 5 x stomach capacity respectively.

log FMR = -0.28 + 0.73.log BM.

log SC = -1.17 + 0.93.log BM.

Similarly, seabirds of masses 794 g and 100 g would require daily volumes of food equivalent to twice and three times their respective stomach capacities.

In an attempt to translate these ingestion rates into total gut passage rates, I performed a similar exercise incorporating hindgut volumes (in cm^3, Chapter 3) into the equation for gut capacity. Hindgut contents were assumed to have a specific gravity of unity, for the purposes of conversion to g (wet mass), and the volume of metabolizable dry matter was estimated in the same way as for stomach contents. The implications of deviations from this assumed specific gravity for flying birds are addressed briefly later. Fig. 7.6 indicates that, assuming the volume and density of digesta remain unchanged throughout the gut, a seabird weighing 8.9 kg would have to replace its entire gut contents once a day in order to process enough digesta to meet its energy needs, and one weighing 602 g would have to do so twice a day. These rates correspond with mean retention times of 24 and 12 hours respectively: observed mean retention times for fish and squid in five seabird species weighing between 2 and 14 kg (Chapter 3), fall well within this range. Obviously, line (B) represents an underestimate of gut capacity in terms of metabolizable energy, because absorption progressively reduces digesta volume in the hindgut, and selective retention of lipids in the stomachs of procellariiforms increases the energy density of proventriculus contents (Chapter 5, Place *et al.*, 1989).

The functions plotted in Figs 7.5 and 7.6 permit estimation of the limits to mean gastric and total gut retention times of digesta, on the basis of mass-specific energy demands and gut capacity. Because absorption was not taken into account, I stress that the exercise was not aimed at predicting gut passage rates in seabirds. Using gut measurements, passage rates and metabolizable energy coefficients presented in previous chapters, the exercise suggests that gut processing capacity probably does not set upper limits on the body sizes of seabirds. This argument is strengthened by my use of generous values for energy requirements (many of the FMRs used in the calculations for Figs 7.5 and 7.6 were measured for birds feeding chicks), and by the possibility that mean retention times in free-living birds may be shorter than values

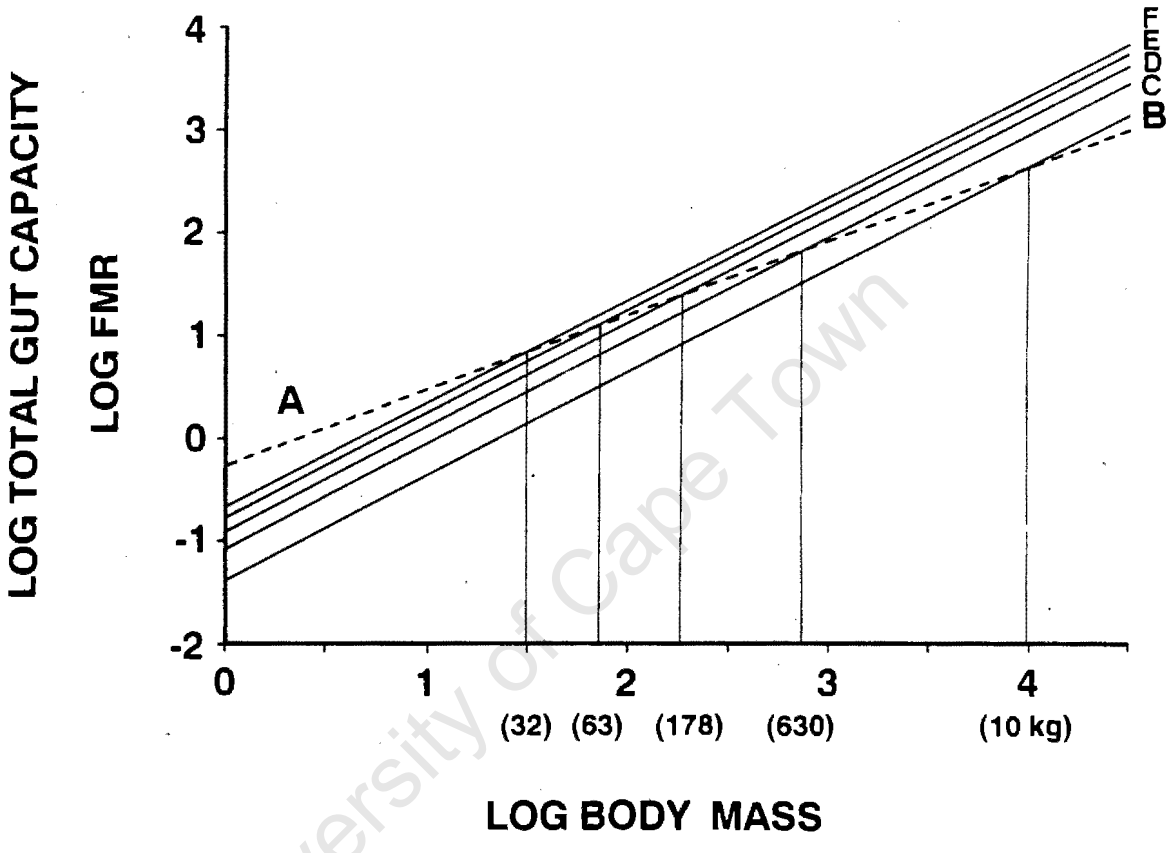

Figure 7.6. Field metabolic rate (g metabolizable dry matter.d^{-1}) and total gut capacity (g metabolizable dry matter, estimated as the sum of stomach and hindgut volume) as functions of body mass. Data obtained from Figs. 7.3 and 7.4. A: FMR, B: hindgut capacity, C, D, E, and F: 2 x, 3 x, 4 x, and 5 x hindgut capacity respectively.

Log HC = -1.39 + 1.01.log BM.

reported in Chapter 3 for single meals, because the more frequent ingestion resulting from natural foraging probably speeds up passage of digesta already in the gut. Comparison of chick and adult energy requirements with the maximum "payload" that can be lifted by seabirds of various sizes might indeed reveal that this aspect of gut capacity limits the foraging efficiency of seabirds (Ricklefs, 1983; Pennycuick *et al*, 1984).

At the lower end of the body-size scale, mass-specific metabolic rate becomes progressively higher in relation to mass-specific gut volume (Figs 7.5 and 7.6). The equation given in Fig. 7.4 indicates that a seabird such as Wilson's Storm-petrel *Oceanites oceanicus*, with a body mass of 42 g (Obst *et al.*, 1987), would have a stomach volume of 11.23 cm^3, and that it would have to fill this nearly four (3.7) times a day (ie: eat its own weight in food per day, Fig. 7.5) to meet its energy requirements*. In fact, a doubly-labelled water study indicates Wilson's Storm-Petrels must eat 120 to 150% of their body mass per day (Obst *et al.*, 1987). The FMR equation given in Fig. 7.4 apparently underestimates the energy requirements of small seabirds, and estimates of the number of stomach volumes needed daily to supply small birds energy needs are thus likely to be conservative.

Mean retention times of aqueous components in Leach's Storm Petrel *Oceanodroma leuchorhoa* stomachs are 0.35 hours (Place *et al.*, 1989), but neutral lipids are retained in the stomach for a mean of 70 h. Recent work on stomach oil formation in procellariiforms indicates that much of the energy metabolized by Wilson's (Obst, 1986) and Leach's (Ricklefs *et al.*, 1986) storm-petrels is in the form of stomach oils. These are of a far higher energy density than that assumed in the calculations for Fig. 7.6, and total gut volumes needed to fuel storm-petrel energy requirements are consequently much lower than those predicted in Fig 7.6. For seabirds apparently reaching the lowest possible limits of body size, the energetic

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*The proventricular volume of oil retained by Leach's Storm-petrel Oceanodroma leucorhoa chicks (ca. 8 ml, Place et al., 1989) is close to the stomach volume of 10.5 ml predicted using the equation given in Fig. 7.3, indicating that the regression yields plausible values for stomach volumes.

advantages conferred by stomach oil formation (Ricklefs, 1974; Roby *et al.*, 1989) may well be crucial to survival and reproductive success. The relationship between gut capacity and metabolic rate may well be a factor influencing the lower body size limits for seabirds. The disadvantage of a decrease in mass-specific gut capacity may be offset by improved assimilation efficiency resulting from an increased surface area to volume ratio in small seabird guts.

The partitioning of body mass or space between digestive organs and flight muscles is an intriguing aspect of avian functional morphology that has not to my knowledge been addressed in the literature. Aerodynamic principles dictate that the power required from flight muscles increases with body mass$^{0.17}$, whereas the mechanical properties of muscle result in a decrease in muscle power output with increasing body mass, as muscle power output scales with body mass$^{-0.33}$ (Pennycuick, 1975). However, the mass of flight muscles does not appear to be directly correlated with bird body mass (Greenewalt, 1962). Large birds thus have proportionally smaller engines yet larger fuel tanks (guts) than small species! This is an apparent paradox that might be solved by anatomical and physiological studies of birds at extremes of the body-size range.

Gut surface area

The link between gut surface area and assimilation efficiency is an intuitively obvious one, and has been demonstrated for reptiles and mammals (Karasov and Diamond, 1985). Furthermore, the net amount of energy absorbed from food in the gut should increase with increasing retention time, up to such time that the rate of energy gain is exceeded by the cost of digestion (Sibly, 1981). If seabird assimilation efficiency is a constant optimal value, as it seems to be except during times of extreme energetic stress such as moult, one might expect that fast passage rates in seabirds should compromise assimilation efficiency. Do seabirds with faster gut passage rates have larger gut surface areas, to compensate for the decrease in time available for absorption? This is apparently not true of the Cape Gannet, however, which exhibits the fastest passage rate of all species studied. As suggested in

Chapter 4, the measurements of hindgut surface area presented here may not be detailed enough to detect the influence of this parameter on assimilation efficiency in seabirds.

Seabirds: digestive specialists or opportunists?

Can seabirds afford to have specialized digestive systems, or would this compromise their ability to exploit unpredictable prey opportunistically? Of the functional digestive parameters investigated, energy assimilation efficiency appears constant at the apparently optimum level of 75%, and is not influenced by prey type. Seabirds' abilities to digest and assimilate substances specific to certain prey, ie: chitin and wax esters, may reflect dietary specialization and are shared within families, but differ between the three groups studied (Procellariiformes, Spheniscidae and Sulidae). Procellariiform stomach stucture shows distinctive morphological adaptations that facilitate foraging over wide areas for sometimes unpredictable prey. Hindgut structure influences gut retention times, and may reflect adaptations to the different lifestyles of penguins and volant seabirds. Body mass or size has a profound influence on digestive tract structure and function, and, because size influences prey selection, on dietary specialization. For these reasons, future studies of seabird digestive processes may benefit by employing an allometric approach. Seabirds exhibit digestive specializations reflecting the diverse foraging methods employed by different families. Within families, individual species are opportunists capable of digesting fish, squid and crustaceans with similar efficiency.

This study has shown that seabirds show interspecific specialization with respect to their ability to assimilate energy from different prey types. This accords with the predictions of the "patchy prey" hypothesis and supports the idea that seabirds are opportunistic foragers. However, constraints imposed by central place foraging during the breeding have had major effects on the evolution of gut morphology, especially on gut length, volume and shape. Differences in gut morphology between species are better explained by differences in foraging technique and range rather than by differences in diet.

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APPENDIX 1

STOMACH PUMPING: IS KILLING SEABIRDS NECESSARY?

Stomach Pumping: Is Killing Seabirds Necessary?

PETER G. RYAN AND SUSAN JACKSON
FitzPatrick Institute, University of Cape Town, Rondebosch 7700, South Africa

Many seabird species regurgitate when handled, allowing diet assessment without killing birds (e.g. see Ashmole and Ashmole 1967, Harrison et al. 1983). Other seabirds, notably penguins (Wilson 1984) and petrels away from their breeding grounds (Harrison et al. 1983, pers. obs.), are less willing to regurgitate. A quantitative, but nonlethal, sampling technique is needed for diet studies on these seabirds, particularly in view of the growing opposition toward the killing of animals for biological research.

Emetics and stomach pumps have been used to obtain stomach contents from seabirds, but results have been unsatisfactory (Wilson 1984, Duffy and Jackson MS). Wilson (1984) described a simple technique for sampling stomach contents in seabirds, but it has been suggested that it does not always recover the entire stomach contents (Lishman 1985; but see Horne 1985) and is less effective on birds that have full stomachs with tightly packed contents (Lishman 1985). We tested the efficiency of Wilson's stomach pump on four species of petrel and review its use in other birds.

Seven White-chinned Petrels (*Procellaria aequinoctialis*) (mean mass 1,250 g) from Marion Island (46°52'S, 37°51'E) each were fed a large meal (125 g) of squid (*Loligo* sp.), lightfish (*Maurolicus muelleri*), and antarctic krill (*Euphausia superba*) in equal proportions, then pumped and killed after varying intervals. The amount (mass and number of prey items) of food recovered by stomach pumping was expressed as a proportion of the total stomach contents (determined by dissecting out the oesophagus and proventriculus) and compared with the total stomach contents. In addition, single Cape Petrels (*Daption capense*), Salvin's Prions (*Pachyptila vittata salvini*), and Wilson's Storm-Petrels (*Oceanites oceanicus*) were collected at sea off southern Africa, then similarly tested.

Mean pump efficiency (the proportion of food recovered by a single pumping) was 89.2% (SD = 13.3) by mass and 99.1% (SD = 2.0) by number of prey items ($n = 10$). The proportion of food (by mass) recovered by a single pumping was negatively correlated with total stomach content mass in the 7 White-chinned Petrels examined (Fig. 1; $r = -0.85$, $P < 0.01$ on arcsine transformed data). The proportion of prey items recovered was also negatively correlated with the total number of items present ($r = -0.67$, $P < 0.05$, $n = 10$). Approximately equal masses and numbers of the three prey types were recovered, irrespective of stomach fullness. When stomachs were less than 20% full, the entire contents were removed by a single pumping. The three other petrel species tested all yielded 100% of their stomach contents.

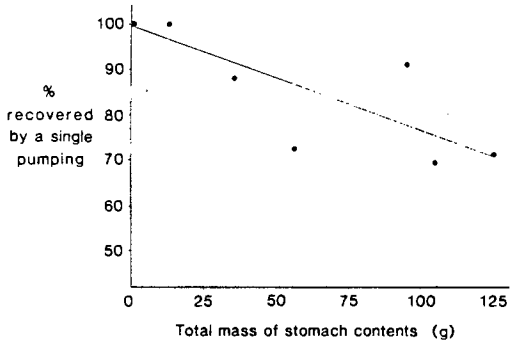

Fig. 1. Stomach pump efficiency as a function of stomach fullness in White-chinned Petrels.

The contents of the petrels' gizzards (ventriculi) were not removed by the pumping technique. This is apparently due to the narrow, U-shaped isthmus between the proventriculus and gizzard in petrels (McLelland 1979). This is not a major disadvantage, because the gizzard seldom contains fresh food items in petrels. Gizzard samples introduce a bias toward resistant prey remains (Furness et al. 1984). The only way to sample petrel gizzard contents accurately is to kill the birds (Furness 1985). In other seabird groups (e.g. Sphenisciformes, Pelecaniformes, Charadriiformes, and to some extent Diomedeidae) the gizzard is less clearly separated from the proventriculus, and its contents may be sampled by the pumping technique.

The high proportion of stomach contents recovered both by mass and by number of prey items indicates the usefulness of the stomach pump in seabird diet studies. Pumping birds that regurgitate when handled ensures that all the stomach contents are removed. If a bird is pumped twice, the entire stomach contents should be removed, even if the stomach is full and tightly packed with food. More than 60 Adélie (*Pygoscelis adeliae*), Chinstrap (*P. antarctica*), and Gentoo (*P. papua*) penguins with stomachs full of crustacean prey have been emptied completely by successive pumping (W. Z. Trivelpiece in litt.), contrary to the objections of Lishman (1985). The technique was tested by killing the first five penguins sampled and was found to be 100% efficient.

The overall efficiency of Wilson's stomach pump is to a large extent dependent on the experience of the operator. Workers without adequate training in the technique are unlikely to obtain representative samples at first. The advantages accruing from the pump's use, however, are great. More than 2,500 seabirds from 24 species (including penguins, albatrosses, petrels, skuas, gulls, and terns) have been pumped with only 1 known mortality, which was due to worker incompetence (FitzPatrick Inst. unpubl. data). Many birds have been sampled repeatedly with no apparent ill effects (R. P. Wilson pers. comm.). Simple modifications using narrower catheter tubes and a syringe as a pump have enabled its successful use on small seabirds such as storm-petrels and diving petrels and other bird groups including waders (Charadrii) (G. D. La Cock pers. comm.). We recommend the use of Wilson's stomach pump as an effective and efficient, nonlethal sampling technique for determining bird diets. Birds should be pumped twice to ensure that the entire stomach contents are removed.

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APPENDIX 2

DIFFERENTIAL DIGESTION RATES OF PREY BY WHITE-CHINNED PETRELS (PROCELLARIA AEQUINOCTIALIS)

Differential Digestion Rates of Prey by White-chinned Petrels (*Procellaria aequinoctialis*)

SUE JACKSON AND PETER G. RYAN
Percy FitzPatrick Institute of African Ornithology, University of Cape Town, Rondebosch 7700, South Africa

We investigated the gastric digestion rates in the White-chinned Petrel (*Procellaria aequinoctialis*), which is a generalist feeder (Croxall and Prince 1980, Croxall et al. 1984, A. Berruti pers. comm., S. Jackson unpubl. data). The natural diet of White-chinned Petrels includes the three prey types used in the experiments: light-fish (*Maurolicus muelleri*), squid (*Loligo reynaudi*), and antarctic krill (*Euphausia superba*) (A. Berruti pers. comm., S. Jackson unpubl. data).

Seven fledgling White-chinned Petrels were removed from their burrows on subantarctic Marion Island (46°52'S, 37°51'E) and kept in separate wire-mesh cages (40 × 40 × 60 cm) for 12 days before the start of the experiment. We maintained the birds at approximately constant mass on diets of equal proportions of light-fish, squid mantle flesh and heads, and antarctic krill. We noted the total numbers of squid beaks fed to the birds throughout their period of captivity.

For the experiment, we fed each bird a mixed meal comprising 40 g each of light-fish (approximately 40 individuals), squid (1 head and several pieces of mantle), and krill (approximately 60 individuals). All the food had been frozen, but was thawed slowly and handled with care during feeding to avoid tissue damage that could have affected the rate at which food was digested.

We stomach-pumped individual birds 15 min and 1, 2, 4, 7, 12, and 24 h after feeding, using a water-offloading technique (Wilson 1984). We then killed the birds and dissected their oesophagi, stomachs, and gizzards. The samples were drained and weighed. We counted identifiable food objects separately, noting the apparent state of digestion. We counted all cephalopod beaks recovered and noted their state of wear. We also counted otoliths and krill eye lenses.

The mass of all food types recovered after 15 min increased by less than 5%, presumably due to the water added during stomach pumping, or to initial water absorption by the prey. We compensated with a correction factor (C) such that $C = I/F'$, where I = initial mass of a food type and F' = mass of that food type recovered after 15 min. We assumed that the mass gain of each food type was proportionally the same for the birds stomach-pumped after different time intervals. We expressed the mass of food recovered as a percentage of the mass initially fed to the bird after each time interval.

White-chinned Petrels digested light-fish more rapidly than they digested either squid or krill: no traces of fish remained in the stomach after 12 h (Fig. 1). The results of similar experiments on Jackass Penguins (*Spheniscus demersus*; Wilson et al. 1985) are included for comparison (Fig. 2). We could not count individual fish in White-chinned Petrel stomachs after 4 h (Fig. 3). Initial digestion of krill was slower

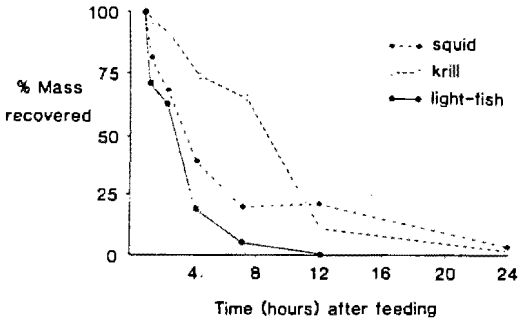

Fig. 1. Percentage of the mass of different foods recovered from White-chinned Petrels at increasing intervals after feeding.

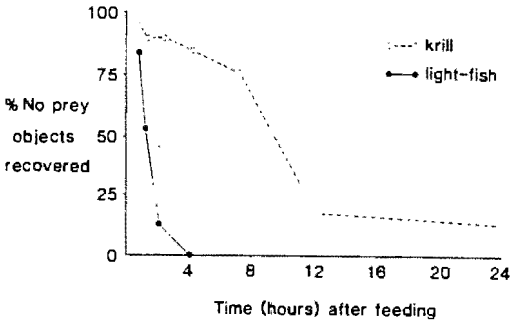

Fig. 3. Percentage of individual objects of different foods recovered from White-chinned Petrels at increasing intervals after feeding (counts of light-fish after 1 h = number of brain cases; counts of krill after 6 h = number of pairs of eyes).

than that of the other food types. After 12 h, however, proportionally more squid remained (Fig. 1). Pieces of squid appeared unchanged after 4 h, but their mass decreased by more than 50%. The number of krill could still be determined after 24 h by counting the loose eyes present in the stomach.

We distinguished three discrete states of wear in the cephalopod beaks: "fresh," with the brittle "wings" (Clarke 1962) intact; "intermediate," with the rostrum sharp but with broken or abraded wings; and "worn," when all surfaces of the beak were smooth and rounded. A mean of 87% of all the *Loligo* sp. beaks recovered from the 7 White-chinned Petrels after 3 weeks of captivity were fresh (range = 62.5–100%). All the birds contained worn beaks of other cephalopod species that must have been ingested before capture.

Short-term adaptations to specialized diets may affect the digestive efficiencies of different individuals of the same species (Partridge and Green 1985). However, interspecific differences in the prey digestion rates of seabird species with different diets have not been documented.

Although White-chinned Petrels show a general pattern similar to that observed in Jackass Penguins, differences in the digestion rates of fish and squid occur. Total passage time of squid through the stomach was similar in both birds, but squid lost mass faster in the White-chinned Petrel. After 4 h, squid remains in White-chinned Petrel stomachs were less than 50% of the mass of those in Jackass Penguin stomachs (Wilson et al. 1985). Initial digestion of light-fish by White-chinned Petrels also may be faster than digestion of anchovy (*Engraulis capensis*) by Jackass Penguins, but the evacuation time of all anchovy remains from the stomachs of the penguins was shorter than that of light-fish from White-chinned Petrel stomachs.

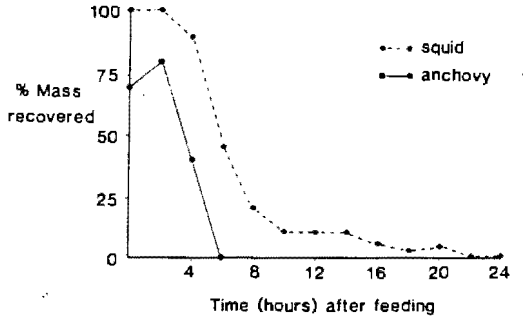

Fig. 2. Percentage of the mass of different foods recovered from Jackass Penguins at increasing intervals after feeding (after Wilson et al. 1985).

The rate of mass loss by squid remains in the stomachs of both White-chinned Petrels and Jackass Penguins decreased after 8 h (Figs. 1 and 2). This possibly was due to the presence of resistant collagen fibers in squid mantle (Gosline and DeMont 1985). Tissue structure of prey may affect the rate at which items are digested, independently of adaptations in the birds to specialized or generalized diets.

The initially slow digestion of krill by White-chinned Petrels probably is due to the chitinous crustacean exoskeleton, which retards penetration by the digestive juices. This effect may be reduced in the wild, where damage to prey during capture may provide sites for entry of enzymes.

The persistence of squid and crustacean soft remains in seabird stomachs may result in overestimation of the relative importance of such food types in seabird diets, as does the persistence of squid hard parts (Furness et al. 1984). Otoliths are digested rapidly in seabird stomachs (Duffy and Laurenson 1983). Consequently, the importance of fish in seabird diets may be underestimated.

Differential digestion rates may be due both to the nature of the prey and to differences in the digestive systems of the predators as a result of dietary specializations. Whatever the causes of differential

digestion in seabirds, allowances must be made for *unequal* retention times of both hard and soft prey remains in seabird stomachs to avoid biases in diet studies.

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